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ORIGINAL ARTICLES

THE THERAPEUTIC USE OF RADIOACTIVE PHOSPHORUS*†‡

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Radioactive phosphorus, first prepared by E. O. Lawrence and utilized by J. H. Lawrence and his associates for the therapy of leukemia[7] and polycythemia,[6] has been tested in several localities. It is still being used in a few places, in spite of the difficulty of obtaining it owing to present exigencies of the war. From two centers there have been reported large series of cases: Those followed by Lawrence and his colleagues at the Crocker Radiation Laboratory[6,7,8] and those treated at the Memorial Hospital, New York, by Kenney,[5] Craver,[1] and their associates. The present group consisting of 81 cases and begun in January 1940 constitutes the third large series to be reported.

Radioactive phosphorus as utilized in this series has been prepared in one of two ways: (1) the bombardment by the cyclotron of red phosphorus placed in the external target chamber, and (2) the bombardment of an iron phosphide probe placed in the deuteron stream of the cyclotron just inside the port.

The first method gives a product of relatively low specific activity; the second, one of high specific activity. By either method, only a fraction of the total phosphorus bombarded is rendered radioactive. Depending on the size of the cyclotron and whether the phosphorus is placed in the target chamber or on a probe, the necessary time of exposure to produce a given radioactivity will vary. It usually requires 6 to 8 hours of bombardment to obtain 25 millicurie equivalents of

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activity under favorable conditions. The phosphorus is converted by standard chemical procedures, first to phosphoric acid and then usually to dibasic sodium phosphate. Chemical manipulation has no effect on the radioactivity, the temporarily radioactive atoms reacting as would any phosphorus atom.

Radioactive phosphorus has a half life of 14.3 days; and, like all radioactive substances, has a steady rate of decay. The reaction of its formation is:

$$_{15}P^{31} + {}_{1}D^{2} \rightarrow {}_{15}P^{32} + {}_{1}H^{1*}$$

P^{32} gives off an electron as beta radiation, thereby being converted to sulphur according to the following reaction:

$$_{15}P^{32} \rightarrow {}_{16}S^{32} + {}_{1}E^{-}$$

While only one of many temporarily radioactive substances there are striking advantages in the use of radioactive phosphorus as a means of internal therapeutic radiation. Its half life is long enough to permit the necessary chemical manipulations to prepare it for administration without undue loss of activity; it is short enough to have no long-range harmful effects on the body. The beta rays given off have relatively low penetrating power (2 to 4 mm. of tissue). Certain cells, among them leukemic cells, preferentially absorb the material. The end-product of the reaction, sulphur, is not radioactive and is harmless.

Methods. The measurements of radioactivity were carried out on a modified Geiger-Muller counter, checked from time to time with a Lauritsen type electroscope. For the measurements of radioactivity, I am indebted to Mr. R. F. Cowing of this hospital. The energy of radioactive phosphorus is measured in millicurie equivalents; that is, the ionizing power of its beta radiation as compared with the ionizing effect on air of 1 millicurie of radon. It will be seen at once, owing to the different types of radiation involved (beta as against beta and gamma), that there can be no direct translation of biologic effectiveness from radioactive phosphorus to radon. It has been calculated that 0.1 microcurie of radioactive phosphorus per gram of tissue delivers about 355 ergs in the first 24 hours.[8] This has the ionizing effect of 4.2 roentgens of therapeutic Roentgen rays delivered per gram of tissue.

At first, we administered P^{32} as Na_2HPO_4; then, as skilled chemical help became less abundant, magnesium ammonium phosphate ($MgNH_4PO_4$) produced as a step in the preparation of sodium phosphate, was utilized and proved satisfactory. As a still further simplification, even H_3PO_4 has been used when the lots have been of high specific activity, without a single untoward reaction.[12] We are indebted to Dr. John Irvine of M. I. T. for these latter forms.

As a rule, the desired dose of P^{32} was administered intravenously dissolved in 300 cc. of 0.85% NaCl and 5% glucose in the case of adults, and in 100 cc. of the same diluent in the case of infants and young children. Glass or rubber tubing of ordinary thickness absorbs a sufficient amount of radioactivity to render special precautions for the protection of personnel unnecessary during the brief period of handling. When the material is given orally, the dose is dissolved in 150 cc. of orange juice. With oral administration, 20 to 30% of the dose is not absorbed, owing largely to precipitation of a portion in the gastro-intestinal tract as insoluble phosphates.

* This arrangement has been adopted by physicists to signify the atomic number in the subscript and the atomic weight in the superscript.—Ed.

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We prefer the intravenous route for giving radioactive phosphorus since this entails accurate measurement of the dose absorbed, conservation of material and the rapid attainment of an adequate concentration in the blood stream. A limited number of cases have been treated with radioactive phosphorus by the oral route as a check against the intravenous method. In most cases, measurements of the concentration of radioactivity in whole blood and its various components were made.[11] The rate of excretion was also followed in the majority of the cases.

We have selected for P^{32} therapy those types of disease known to be highly radiosensitive—leukemia, lymphoma, plasmacytoma and Hodgkin's disease. However, we decided to limit the cases chosen for treatment chiefly to those which after initial favorable response to roentgen radiation have become resistant, and also to those cases of leukemia known commonly to do badly with Roentgen radiation as, for example, acute leukemia in childhood. A few moribund cases of chronic leukemia were treated partly to see if anything could be accomplished for them, partly to learn whether the effect of P^{32} varied from the usual in those cases with extremely high white blood cell counts and very low red blood cell counts. Because of the cases selected for therapy the results in this series will appear discouraging in the aggregate.

We based our decision to work with this relatively unfavorable group on several reasons. The efficacy of P^{32} therapy of chronic leukemia was being evaluated by the careful studies of Lawrence and others. Most cases of chronic leukemia will respond fairly well, at least temporarily, to Roentgen therapy. Particularly at the beginning of the study, P^{32} was difficult to obtain and the supply was somewhat uncertain. It hardly seemed fair to use the material under these conditions for patients who in all probability would do well with more readily available methods of treatment.

There is always discussion as to the classification of leukemias. We have endeavored to classify these cases according to accepted methods. In a few instances where there was a question of classification, blood smears and biopsies of lymph nodes or bone marrow have been submitted to several authorities. Four types of leukemia are included in the series: myelogenous, lymphatic, monocytic and plasma cell. We have attempted to distinguish 3 phases of activity of the leukemic process: acute, subacute and chronic, based upon the proportion of blasts present in the circulating blood and the clinical course of the patient. By no means can all the cases be readily pigeonholed in these groups and certain arbitrary standards were devised. If over 20% of the cells in the circulating blood were blasts at the time the case was first seen and during the initial days of therapy, it was regarded as subacute; if over 50%, acute. The acute cases were all febrile; the subacute cases were usually febrile. Petechiæ, purpuric lesions and hemorrhage from mucous membranes were considerably more frequent in the acute and subacute cases than in the chronic.

The cases were further divided into: (*a*) those treated with Roentgen radiation who had either failed to respond after a period of previous good response or proved to be resistant from the start; and (*b*) those

who had had no previous radiation therapy. There were 31 of the former and 50 of the latter.

TABLE 1.—RESULTS OF THERAPY

Type	Previous Roentgen ray therapy	Number of cases	Number helped	Per cent helped
Chronic myelogenous leukemia	−	9	5	56
	+	10	4	40
Subacute myelogenous leukemia	−	2	2	100
	+	0	0	0
Acute myelogenous leukemia	−	8	0	0
	+	1	0	0
Chronic lymphatic leukemia	−	3	1	33
	+	5	4	80
Subacute lymphatic leukemia	−	2	0	0
	+	2	1	50
Acute lymphatic leukemia	−	8	1	13
	+	4	2	50
Monocytic leukemia	−	4	1	25
	+	0	0	0
Acute leukemia, unclassified	−	8	0	0
	+	0	0	0
Lymphosarcoma	−	2	0	0
	+	2	0	0
Plasma cell leukemia	−	0	0	0
	+	1	0	0
Plasmacytoma	−	0	0	0
	+	3	2	67
Hodgkin's disease	−	0	0	0
	+	3	1	33
Polycythemia vera	−	3	2	67
Blood dyscrasia, unclassified	−	1	1	100
Total		81	27	33

A total of 81 cases were treated in the period from January 1940 to September 1944 (see Table 1). Four (Cases 1 to 4) have been reported in part previously.[9] In this table the number of cases in each category and the number that were helped by the treatment are reported. Those cases were considered helped which showed definite clinical and laboratory evidence of improvement. Such a person regained a fair degree of former activity for a period of 3 months or more, the white blood cell count was significantly lowered with an increase in the proportion of mature forms present, and the temperature returned to normal. The basal metabolic rate, when determined, was lowered. The possibility of spontaneous remission must always be kept in mind, but in the great majority of these cases spontaneous remission would be most unlikely. The best results were obtained in previously untreated cases of chronic and subacute myelogenous leukemia, 7 of 11 showing improvement, and in lymphatic leukemia previously treated with Roentgen ray, 7 of 11 again showing improvement. The cases of leukemia in childhood in general did badly, although some promising leads were obtained. These cases, most of which were studied in collaboration with Dr. Louis K. Diamond, will be reported in detail later. Histories of representative cases are presented below.

Case Histories. CASE 7. R. K. *Chronic myelogenous leukemia, advanced but not terminal, previously untreated.*

Patient, a married white female, 45 years of age, entered the Peter Bent Brigham Hospital November 14, 1940, with a history dating back to 1934 when cholecystectomy was performed, followed by choledogastrostomy and subphrenic abscess, then for several years recurrent attacks of cholangitis with development of a biliary cirrhosis. In summer of 1940, she had severe left abdominal pain for 10 days (relieved by binder) followed by abdominal fullness and 20 pounds weight loss. Severe left upper quadrant pain 2 days before admission. Patient was moderately obese, with a few soft, palpable cervical nodes; spleen extended to right of and 10 cm. below umbilicus; friction rub and tenderness over spleen; liver 6 cm. below costal margin; slight edema of ankles. White blood cell count was 322,000, red blood cell count 3,550,000, hemoglobin 9.9 gm., neutrophils 40%, band forms 22%, myelocytes 33%, myeloblasts 2%, basophilic leukocytes 2%, eosinophils 1%. Sternal bone marrow biopsy showed myelogenous leukemia. P^{32} therapy (in the form of NaH_2PO_4 dissolved in 0.85% NaCl and 5% glucose) started with 2260 μc. intravenously on Nov. 20, 1940, then 2600 μc. intravenously on November 30, which was followed by a chill. By December 29, the spleen was somewhat smaller and patient had improved clinically. Patient returned home and was readmitted Feb. 4, 1941, generally stronger; spleen 10 cm. and liver 2 cm. below costal margins; white blood cell count 44,000, red blood cell count 3,620,000, platelets normal; 3190 μc. P^{32} intravenously given on Feb. 5, discharged condition unchanged. By March 20, white blood cell count 14,400, red blood cell count 3,930,000 and normal differential except for 7% myelocytes, basal metabolic rate +9, spleen extended 4 cm. down and liver not enlarged; 1000 μc. intravenously given. White blood cell count varied between 6800 and 11,000 through July 1941; patient felt well and spleen was practically normal. On September 15 white blood cell count 27,000 with 70% band forms. General condition good, but spleen extended to umbilicus; 3500 μc. P^{32} given intravenously. White blood cell count fluctuated between 36,000 and 71,000 until April 1942, with spleen extending below umbilicus and liver 6 to 10 cm. below costal margin. Received 2600 μc. intravenously on Nov. 24, 1941, and 1500 μc. on March 10, 1942. Readmitted on April 2, acutely ill, dyspneic, weak, with dullness in both lung bases, distended abdomen, spleen 8 cm. below umbilicus and liver 10 cm. below costal margin. White blood cell count 320,000 with 66% blasts, platelets normal. Received 1620 μc. P^{32} intravenously on April 3 and 2140 μc. on April 6. Patient died April 8 and autopsy (A-42-51) showed myelogenous leukemia involving heart, lung, spleen, liver, pancreas, kidneys, uterus, ovary, lymph nodes, bone marrow (chloroleukemia); pulmonary atelectasis; cerebral edema; biliary cirrhosis.

Comment. This was a favorable case that should have done better. The treatment was probably inadequate after February 1941.

CASE 60. F. Z. *Terminal chronic myelogenous leukemia, previously radiated and found resistant.*

Married white woman, 47 years of age. Developed symptoms of anemia in April 1941, and was found to have an enlarged spleen and chronic myelogenous leukemia. In spite of repeated Roentgen ray therapy her response was unsatisfactory and her white blood cell count remained well elevated. April 13, 1942, her white blood cell count was 250,000, her red blood cell count 3,770,000, hemoglobin 9.5 gm. Her differential count showed neutrophils 56%, band forms 12%, myelocytes 25%, myeloblasts 1%, lymphocytes 2%, eosinophils 2%, basophils 2%; platelets normal.

Her general condition was fair. The spleen occupied the entire left side of abdomen. On April 17, 1942, she was given 1800 μc. intravenously without reaction. The basal metabolic rate was +34. April 23 she was given a second dose of 1800 μc. intravenously, on May 12, 2700 μc. intravenously, on July 8, 1400 μc. intravenously, August 14, 2120 μc. orally. Her general condition had materially improved, and the spleen had diminished to about two-thirds its former size. The white blood cell count had remained fairly high;

it did not go below 100,000. October 31 she was given 2800 μc. orally and on November 21, 1800 μc. She was active, doing her own housework and had no complaint except "heaviness" due to a prolapsed uterus. Jan. 5, 1943, she was given 3800 μc. of radioactive phosphorus intravenously and on January 8, 1600 μc. intravenously. The spleen was 3 cm. below the level of the umbilicus. The white blood cell count was 82,400, red blood cell count 3,830,000. February 9 she was given 1900 μc. orally. March 11 she was given 1600 μc. intravenously and March 12, 1800 μc. intravenously. On April 22 she was given 2700 μc. intravenously. Her general condition continued satisfactorily. Her white blood cell count at this time was 159,500, red blood cell count 4,070,000, hemoglobin 11.8 gm.; platelets normal. She continued doing fairly well until June 4. She had complained of some weakness and pain and numbness in right thigh. She was given 1800 μc. orally. August 4 she had developed a marked anemia with a red blood cell count of 2,830,000, and on September 1 it had fallen to 1,960,000. She was given a 500 cc. transfusion of citrated blood. Her general condition deteriorated rapidly and it became apparent that further therapy would be useless. November 6 she died. Diagnosis: chronic myelogenous leukemia.

Comment. This case was one which had failed to respond satisfactorily to Roentgen therapy and was virtually moribund when treatment was attempted. She was given rather light doses of radioactive phosphorus at first and responded well clinically, maintaining a satisfactory red blood cell count and being able to go about her normal duties. Her white blood cell count never reached satisfactorily low levels and her spleen remained considerably enlarged in spite of her general improvement. After 15 months she developed a rapidly progressive anemia. This case had a year and a quarter of activity added to her life although there was never adequate control of the leukemia by radioactive phosphorus.

Case 63. E. B. *Terminal chronic lymphatic leukemia.*

Patient, a married white male, 49 years of age, entered New England Deaconess Hospital June 4, 1942, complaining of enlarged lymph nodes of 2½ months duration and gradually increasing weakness for some months previously. He had recently received two 500 cc. citrated blood transfusions at the Boston City Hospital. Examination showed axillæ filled with hard, discrete, rounded nodes up to 10 cm. in diameter. Scattered, somewhat smaller nodes in both groins and numerous firm nodes up to 3 cm. in diameter in the neck, particularly on the left. Basal metabolic rate +50; white blood cell count 296,000, red blood cell count 4,030,000, hemoglobin 12.3 gm. Differential count at this time was: neutrophils 3%, lymphocytes 75%, young lymphocytes 21%, eosinophils 1%, blood platelets markedly decreased.

June 6, 1942, the patient was given 3900 μc. of radioactive phosphorus intravenously followed on June 18 by 1800 μc. and on June 26 by 3000 μc. intravenously. July 27 he was given 600 μc. of radioactive phosphorus intravenously. His white blood cell count was 28,950, hemoglobin 13.9 gm. His lymph nodes had decreased materially in size, his general condition was satisfactory, and the patient had returned to work as a janitor.

September 12, 1942, he was given 1500 μc. of P^{32} orally and on October 31, 3500 μc. orally and on December 5, 2300 μc. P^{32} orally. On March 27, 1943, he received 1800 μc. intravenously. Cervical nodes were now barely palpable. There were 2 nodes about 3 cm. in diameter in the left axilla. The lower pole of the spleen was palpable. His white blood cell count was 97,000; hemoglobin 14.8 gm. June 5 he was given 1880 μc. radioactive phosphorus intravenously and September 4, 3200 μc. intravenously; October 2, 2000 μc. orally. This latter time the right inguinal nodes were somewhat enlarged. Patient was working steadily.

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Feb. 5, 1944, patient was given 3000 μc. orally. There were nodes in the cervical region and in both groins up to 1 cm. in diameter. The lower border of the liver was at the umbilicus. The spleen extended 5 cm. below the costal margin. The abdomen contained some fluid and there was a palpable epigastric mass. March 10, patient was given 1800 μc. intravenously and on June 17, 4300 μc. orally. August 19 patient received 2200 μc. orally. Felt well, and had been working steadily for more than 2 years. Spleen extended to the anterior superior iliac spine and to the midline. White blood cell count was 172,000; hemoglobin 14.5 gm.

Comment. This is an advanced case of chronic myelogenous leukemia that experienced practically complete relief of symptoms and showed marked regression of leukemic enlargement of lymph nodes, liver and spleen. He has been able to work steadily and has maintained a satisfactory red blood cell count and hemoglobin with fair control of the white blood cell count. The past few months his white blood cell count has risen in spite of fairly heavy therapy.

CASE 51. P. G. *Acute monocytic leukemia.*

A white married male, 31 years of age, was admitted to the New England Baptist Hospital, Dec. 4, 1941, with history of progressive weakness, anorexia, dyspnea and 10 pounds weight loss for 2 months. Patient appeared cachectic, with sallow skin and numerous small nodes in cervical, postauricular and axillary regions. Liver 4 cm. below costal margin. White blood cell count 6500, red blood cell count 2,690,000, hemoglobin, 7.05 gm., neutrophils 3%, band forms 6%, lymphocytes 39%, monoblasts 50%, young lymphocytes 2%, platelets 164,000, reticulocytes 0.3%. Sternal biopsy showed acute leukemia. Started on P^{32} therapy Dec. 5, with 2760 μc. intravenously, then 2900 μc. intravenously on December 13 (the latter followed by slight chill and nausea). Symptomatically the patient was somewhat improved and was discharged December 15, but was readmitted December 24 critically ill, with palate and uvula ulcerated, clotted blood on gums, scattered petechiæ and the spleen palpable. White blood cell count 3500, red blood cell count 1,760,000. Received 615 μc. P^{32} intravenously on December 26. Patient's course continued rapidly downhill with white blood cell count 3500 and red blood cell count 1,550,000 on December 30 (despite 4 transfusions). Differential count on December 29: neutrophils 1%, band forms 1%, lymphocytes 14%, atypical cells 15%, monoblasts 69%. Patient died Jan. 1, 1942.

Autopsy showed acute monocytic leukemia with infiltration of heart, lung, trachea, gums, spleen, pancreas, liver, adrenal, intestine, stomach, appendix, kidneys, prostate, testes and skin; hypoplasia of bone marrow; lipoid histiocytosis of spleen; ulcerated and necrotic lesions of lips and gums; foci of cystic degeneration of brain.

Comment. This fulminating case of acute monocytic leukemia showed no response to therapy.

CASE 61. J. C. *Acute leukemia (unclassified).*

This patient, white male, 3 years of age, was a characteristic case of acute leukemia in childhood with petechial hemorrhages and enlarged lymph nodes. Patient was febrile, acutely ill, and anemic. April 21, 1942, patient was admitted to Children's Hospital with white blood cell count 7750, red blood cell count 1,860,000, hemoglobin 6.6 gm.; platelet count 44,000. Differential count showed blasts 60%, neutrophils 2%, lymphocytes 18%, monocytes 2%, eosinophils 2%, myelocytes 16%.

He was given 3 transfusions, and beginning April 28 he was given 5 daily doses of P^{32} intravenously, totaling 1300 μc. He was transfused on May 4 and 5. On May 12 he showed a white blood cell count of 4750, red blood cell count of 4,070,000, hemoglobin 9.5 gm. Blasts had dropped to 26%. Begin-

ning May 13 he was given 1800 μc. intravenously in 3 daily doses. On June 1 he was given 1000 μc. divided into 2 portions. He rapidly lost ground and died June 7, 1942.

Autopsy showed extensive leukemic infiltration of various organs with some hypoplasia of leukemic bone marrow.

Comment. This was a case of acute, rapidly progressive leukemia in childhood. The course of the disease was not altered by therapy.

CASE 47. B. S. *Plasma cell myeloma.*

The patient, a single white female, 66 years of age, entered the Evans Memorial unit of The Massachusetts Memorial Hospitals, Nov. 10, 1941, with poor healing of dental incisions of gum and gave a history of vague pain, anemia and lethargy of about 1 year's duration. Roentgen ray studies showed the whole skeleton to be extensively involved with multiple myeloma. The liver was 10 cm. below costal margin. The red cell count was 2,300,000; the white blood cell count was 8450, with 6% plasma cells; platelets 218,000, serum protein 10.02%. Sternal puncture showed masses of plasma cells.

She received 1950 μc. radioactive phosphorus intravenously Nov. 12, 1942, and 2500 μc. intravenously November 21. The bone pain disappeared promptly. By the time of her discharge from the hospital on November 25 the macrocytosis had decreased from 120 to 95 c.μ. She showed subjective improvement and on Dec. 22, 1941, was given 2140 μc. intravenously. On January 21, 1942, she received 2140 μc. intravenously and on February 19 she received 2200 μc. At this time her basal metabolic rate was +13. She had some headache. April 14 she was given 1150 μc. intravenously. Roentgen examination showed some recalcification of the osteolytic foci. The red blood cell count was 4,300,000, hemoglobin 12.3 gm., white blood cell count 5600. She had been suffering from hypertension and developed rational hemorrhages in July 1942.

She was readmitted September 24, and received 1600 μc. intravenously. This time her red blood cell count was 4,200,000, her white blood cell count 5800 with 70% neutrophils, and no plasma cells. The platelet count was 552,000; the serum protein 8.76% with albumin-globulin ratio of 0.86:1. Aside from hypertension she was doing well.

Patient was readmitted Feb. 9, 1943, in poor condition. She had had several severe nosebleeds and bone pain reappeared. Her red blood cell count was 1,800,000 and the serum protein 9.46% with albumin-globulin ratio of 0.46:1. Roentgen examination of the bones showed no change. She was given 2300 μc. of P^{32} intravenously. On March 10 the red blood cell count was 1,630,000. Investigation of recent digestive difficulties showed an active duodenal ulcer. March 25 she was given 2 transfusions each of 500 cc. citrated blood and 1830 μc. of P^{32} was given intravenously. Her general condition improved markedly and remained satisfactory for about 2 months, then her weakness returned.

On July 31, 1943, she was given 3940 μc. radioactive phosphorus intravenously. Her red blood cell count was 2,400,000 following 3 transfusions. In September her condition had become serious with weight loss and weakness. She was readmitted to the hospital September 15 and after 5 transfusions her red blood cell count reached 2,010,000. November 16 she was readmitted critically ill with pneumonia of 1 week's duration. Her white blood cell count was 1550, red blood cell count 1,630,000. Her non-protein nitrogen was 65 mg. per 100 cc. She died Nov. 20, 1943.

Autopsy showed multiple myeloma of almost all bones involving also lymph nodes, liver and kidneys. Immediate cause of death was lobar pneumonia.

Comment. This case of multiple myeloma showed appreciable improvement lasting from November 1941 to February 1943. Her condition was complicated by hypertension, nephritis, and terminal lobar

pneumonia. No permanent control of the myeloma was achieved, although its extent was retarded.

CASE 45. S. D. *Polycythemia vera.*

The patient, a single white female, 44 years of age, was admitted to the New England Deaconess Hospital Nov. 25, 1941. She had noticed an increasingly ruddy complexion becoming purple when cold, frequent headaches, and intermittent leg pains. Physical examination was negative except for ruddy color.

Her red blood cell count was 8,680,000, hemoglobin 18.15 gm., reticulocytes 1.2%. Her white blood cell count was 23,950 with 80% polymorphonuclear leukocytes; platelets, 694,000. Nov. 26, 1941, the patient received 3000 μc. radioactive phosphorus intravenously. On Jan. 13, 1942, her red blood cell count was 7,550,000, white blood cell count 8750. She showed no reticulocytes. She was given 1940 μc. of P^{32} intravenously. She had become symptom-free by this time and since then has had no difficulty. On March 30, 1942, her red blood cell count was 6,330,000 with 0.5% reticulocytes. Her white blood cell count was 8750. She was given 1200 μc. of radioactive phosphorus. July 8 her red blood cell count was 4,520,000; her hemoglobin was 12.6 gm.

She has had no further symptoms and no treatment was given until June 5, 1943. At this time her red blood cell count was 5,980,000. She was given 1240 μc. of P^{32} intravenously. March 10, 1944, she was symptom-free with a red blood cell count of 5,980,000; hemoglobin of 16.35 gm. She was seen May 9 with no symptoms from polycythemia but some pain in the left ankle which cleared promptly with rest.

Comment. This patient was relieved of her symptoms a month following her first injection of radioactive phosphorus and has had no recurrence of them in nearly 3 years. We have allowed her red blood cell count to remain a little high. Her disease has been readily controlled with moderate doses of radioactive phosphorus at long intervals.

Discussion. On the basis of the foregoing cases two facts become apparent. First, no harm has been done by the administration of radioactive phosphorus in the doses used. Second, a limited number of cases have done better than would be expected with the ordinary means of therapy. Much hinges on the extent of involvement of the bone marrow by the leukemic process when therapy is undertaken. If there is extensive leukemic infiltration of the marrow and very little normal hematopoiesis, nothing is to be gained by treatment aimed at wiping out the leukemic cells. One will be simply in effect changing a leukemia into an aplastic anemia. Hence a sternal bone marrow aspiration or biopsy is desirable in assessing a case being considered for P^{32} therapy.

That therapeutic doses of radioactive phosphorus do not appreciably damage normal red cell formation is shown by several of the cases which had rapid and well-defined increases in red blood cell count at a time when the white count was dropping following administration of the material. Of interest in this connection is the fact that a similar or smaller dose used in the therapy of polycythemia vera may induce a marked and rapid drop in red cell count.

Apparently if there is some normal marrow left behind, even heavy dosages of radioactive phosphorus will not do permanent harm. Thus in Case 21 very heavy dosage was used in a desperate attempt to ameliorate her condition. The total white count was dropped to a level of

350 but there was a prompt improvement from this low level and the patient had a remission of some months duration following this course of therapy. Therapy of so drastic nature is not to be recommended, however, and is cited simply to show that even heavy dosage will not produce complete destruction of all granulopoietic tissue.

Interestingly enough, the megakarocytes are apparently relatively unharmed by the dosage used and frequently an increase in platelet count follows therapy with radioactive phosphorus. In Case 23, the increasing platelet count was a guide to the beginning of the remission and a falling platelet count antedated the clinical and hematologic recrudescence of the leukemic process.

The basal metabolic rate is one of the useful guides to progress of the disease. This shows to an appreciable degree the activity of the leukemic process and may be an even better index at times during the period of therapy than is the peripheral white blood cell count, which may remain low while the leukemic process is active in the tissues. In several of the cases in the present series, the failure of appearance of a significant decrease in the basal metabolic rate was indicative of the later unfavorable course.

Radiation sickness was encountered only in 1 case (No. 48), even though many of the cases treated had previously been made severely ill by Roentgen radiation. In the early stages of the work, febrile reactions occurred with some of the cases where the specific activity of P^{32} was low and where a sufficient degree of purification of the Na_2HPO_4 had not been carried out. With material of higher specific activity or with adequate purification, no febrile reactions have been noted. One patient (Case 48) complained of nausea following treatment and refused further therapy.

Studies of distribution of radioactive phosphorus in the tissues of these leukemic patients and of its partition in the blood in general confirm the results reported by Erf[2] and others.[3,4,10] Owing to the ready diffusibility of the phosphate ion, P^{32} permeates all the body tissues and fluids but varies strikingly in its concentration. Leukemic tissue picks up a considerably higher concentration of P^{32} than normal tissues.

During the first few hours after the administration of P^{32} it remains in fair concentration in the blood plasma but is readily taken up by the blood cells and the tissues. After some hours much of P^{32} present in the blood stream is in the leukocytes with very little in the plasma and a fair amount in the red cells. Thus in Case 32 an injection of 2120 μc. radioactive phosphorus showed at the end of 2 hours 2.25 μc. per 100 cc. of plasma, 11.3 μc. per 100 cc. of red cells, 22.5 μc. per 100 cc. of white cells. At the end of 24 hours there was 0.7 μc. per 100 cc. of plasma, 12 μc. per 100 cc. of red cells, and 22 μc. per 100 cc. white cells.

At the end of 3 days about 75% of the intravenously administered dose is retained in the body, and 50% at the end of a week.[3,12] The proportion retained is fairly constant.

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While the degree of leukemic infiltration of a given tissue largely influences the amount of P^{32} absorbed, certain tissues tend to concentrate the material. The liver, the kidneys, the spleen, the bone marrow show proportionately large amounts, while cartilage, fat and brain show little. After some days, the concentration in bone itself increases relatively.

Soon after administration saliva contains an amount almost comparable to that in the blood, and this gradually disappears. The normal spinal fluid does not attain a concentration approaching that of the blood until a number of days have passed, when the amount in the blood is low. Bile, particularly several days after injection, may contain larger amounts than does the blood owing to the selective deposition of P^{32} in the liver.[10]

Practically all radioactive phosphorus administered intravenously is excreted by the kidneys. If administered orally, up to 25% may be recovered from the feces, but this is chiefly material which has been precipitated as insoluble phosphates and not absorbed rather than actual excretion. The presence of appreciable amounts of P^{32} in the feces when the material has been administered intravenously indicates either hemorrhage into the intestinal tract or fairly extensive leukemic infiltration of the mucosa.

Summary and Conclusions. 1. Eighty-one cases of leukemia, myeloma, Hodgkin's disease and polycythemia vera have been treated with radioactive phosphorus.

2. Most of the cases had failed to respond to or were known to be refractory to Roentgen therapy or were far advanced.

3. Radioactive phosphorus provides a safe means of internal radiation, somewhat selective, of leukemic tissue.

4. The treatment is palliative rather than curative.

5. It may be temporarily effective in some cases that no longe. respond to Roentgen therapy.

6. It is useless when but little normal hematopoietic tissue remains.

7. Radioactive phosphorus rarely produces radiation sickness.

8. About one-third the cases treated showed temporary improvement.

I am indebted to Drs. William B. Stevens, Louis K. Diamond, Joseph F. Ross and William Dameshek for aid in the clinical management of many of the cases reported here; and to Mr. Russell F. Cowing for determinations of radioactivity.

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RADIOACTIVE PHOSPHORUS IN THE TREATMENT OF POLYCYTHEMIA VERA

Results and Hematologic Complications*

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Radioactive phosphorus, supplied through the courtesy of Dr. J. H. Lawrence of the University of California, has been employed by us as a therapeutic agent in 12 cases of polycythemia vera in $2\frac{1}{2}$ years. It is the purpose of this paper to report the results obtained and the complications encountered with this type of therapy.

Dose and Method of Administration. The intravenous method of administration was employed. The initial dose varied from 4 to 7 mc. in the majority of cases, and second or third injections were given during ensuing weeks whenever the material was available. As circumstances beyond our control prevented us from obtaining radioactive phosphorus at regular intervals, the treatment in some cases was inadequate.

Of the 12 patients treated with radioactive phosphorus, 7 were men and 5 were women. They ranged in age from 41 to 73 years. Of the 12 patients, 11 were known to have had the disease for from 6 months to 13 years and each had been treated by other means (venesection, oral administration of phenylhydrazine, or Roentgen irradiation over the spleen and bones) prior to administration of radioactive phosphorus. The diagnosis in the remaining case was made 2 weeks before administering the substance. In this case, 1500 cc. of blood was withdrawn in 2 weeks.

During the periods the patients were under observation, examinations of the blood were made at intervals of 1 month, and on recurrence of the polycythemia additional radioactive phosphorus was administered. In a few instances, when the material was not available, it was necessary to revert to other methods of treating the disease. The period of observation after treatment with radioactive phosphorus was started varied from 5 to 26 months.

Results. Satisfactory remissions were induced in 8 of the 12 cases and incomplete remissions occurred in 2 cases. In the 2 remaining cases the results were unsatisfactory owing, we believe, to inadequate treatment (Table 1).

Table 1.—Results of Treatment of Polycythemia Vera With Radioactive Phosphorus

Results	Cases
Satisfactory remission	8
Improved; remission incomplete (dose probably inadequate)	2
Unsatisfactory (dose inadequate)	2

* Read by title at the meeting of the Association of American Physicians in Atlantic City, May 9, 1944.

† Since this work was undertaken, Dr. Watkins has entered the armed forces and is now a Captain (MC) USNR.

In the group in which satisfactory remissions were induced, both clinical and hematologic improvement was noted. Complete relief of symptoms attributable to the polycythemia occurred. Up to the time of the present writing, the remissions have lasted from 8 to 26 months. Six of the remissions have had a duration of more than 1 year.

Considerable variation in the doses required to induce satisfactory remissions were noted in this group (Table 2). In the average case of polycythemia vera, Lawrence and his associates[4] originally recommended doses of 14 mc., given in 2 equal injections 3 weeks apart of 7 mc. each. However, it was found that in many cases of polycythemia vera, presumably in cases of milder forms of the disease, smaller doses produced a satisfactory response. In Case 4, in which the patient, a woman 41 years of age, who had had polycythemia vera in mild form for 5 years, there was a remission that lasted 16 months after a single injection of only 4 mc. of radioactive phosphorus. In Case 5, in which the patient was 73 years of age, and who also had mitral stenosis and recurrent attacks of congestive heart failure, definite improvement was produced by the administration of 5 mc. of the substance. On the other hand, the patient who obtained a remission of 26 months duration (Case 1) received a series of 3 injections in a period of 2 months the dose totalling 13 mc. A fourth injection of 2.68 mc. was given 16 months after the third injection even though the polycythemia had not recurred.

TABLE 2.—TREATMENT OF POLYCYTHEMIA VERA WITH RADIOACTIVE PHOSPHORUS (P^{32})

Case	Courses of treatment	No. of injections	Dosage (mc.)	Total dosage (mc.)
1*	1	3	13.00	
	16 mos. later	1	2.68	15.68
2*	1	2	14.00	14.00
3*	1	1	7.00	7.00
4*	1	1	4.00	4.00
5*	1	2	5.00	5.00
6*	1	2	9.36	9.36
7*	1	3	10.60	10.60
8*	1	1	7.05	
	2 mos. later	1	5.25	12.30
9†	1	1	6.00	
	5 mos. later	1	6.60	12.60
10†	1	2	14.00	
	17 mos. later	1	6.40	20.40
11‡	1	1	4.76	
	9 mos. later	1	6.05	10.81
12‡	1	1	2.02	2.02

Character of remission: * Satisfactory.
† Incomplete (clinical improvement).
‡ Unsatisfactory.

In the 2 cases in which the remissions were incomplete, definite clinical improvement was noted but the polycythemia, although improved, was not completely and adequately controlled. Both patients had severe forms of the disease. In Case 9, an initial injection of 6 mc. was given and 5 months later a second injection of 6.6 mc. was administered. Although the erythrocyte count dropped to 5,400,000

per c.mm. 1 month after the second injection, it rose to 7,800,000 4 months later, necessitating the use of venesections to control the disease. In Case 10, an initial course of 14 mc. was given. Five months later the erythrocyte count was 5,680,000 and the hematocrit value was 59%. One month later the erythrocyte count rose to 7,660,000 and it was necessary to employ repeated venesections to control the disease. Nineteen months after the first course of radioactive phosphorus was given, an additional 6.4 mc. was administered. Sufficient time after the last injection of radioactive phosphorus has not elapsed to determine what effect this will have on the disease. The total dose administered to this patient amounted to 20.4 mc.

In the group in which remissions were not obtained there were 2 cases (Cases 11 and 12). In Case 11 the patient was a woman, aged 59 years, who had had the disease in severe form for 13 years. An initial injection of 4.76 mc. did not affect the course of the disease materially and resumption of therapy by means of venesection and the oral administration of phenylhydrazine hydrochloride was carried out. Nine months later an injection of 6.05 mc. was given, but sufficient time has not elapsed after the second injection to allow conclusions to be drawn concerning the effect of the substance on the disease. In Case 12 the patient received only 2.02 mc., a dose we believe to be totally inadequate for the patient. This dose did not significantly alter the course of the disease.

Complications. No toxic reactions developed in any of the cases in which radioactive phosphorus was administered. Radiation sickness did not occur. However, hematologic complications in the form of anemia, leukopenia and thrombocytopenia were noted in several cases and acute leukemia developed in 1 case (Table 3).

TABLE 3.—HEMATOLOGIC COMPLICATIONS OF TREATMENT OF POLYCYTHEMIA VERA WITH RADIOACTIVE PHOSPHORUS (P^{32})

Complications	Cases	Months after administration	Hematologic data*
Anemia	5	2–25	Hemoglobin: 9.8–10.9 gm. Erythrocytes: 3,400,000–4,000,000
Leukopenia	5	½–6	Leukocytes: 2200–4300 (less than 4000 in only 2 cases)
Thrombocytopenia	4	1–2	Platelets: 29,000–86,000
Acute leukopenic myelogenous leukemia	1†	16	Hemoglobin†: 6 gm. Erythrocytes: 2,000,000 Leukocytes: 1200 Platelets: 9000 Myeloblasts: 8%

* The values for hemoglobin are for 100 cc. of blood; those for erythrocytes, leukocytes and platelets per cubic millimeter of blood.

† Hematologic data at time of last dismissal from clinic.

Anemia was observed in 5 cases, and was found to be of the hypochromic or normochromic, normocytic types. In 1 case it developed 2 months after administration of the last injection of radioactive phosphorus; in the other 4 it occurred in from 7 to 25 months after the

administration of the substance. In all cases it was mild and of relatively transient duration.

Leukopenia was encountered in 5 cases also, but in only 2 cases was the leukocyte count found to be less than 4000 cells per c.mm. In 1 case the leukocyte count fell to 2200, two weeks after the last injection of radioactive phosphorus had been given; in the other case, it dropped to 3600 three weeks after administration of the material. The leukopenia was of short duration in all cases, and there were no serious complications as a result of it.

Thrombocytopenia occurred in 4 cases, the platelet counts varying from 29,000 to 86,000 per c.mm. The only hemorrhagic phenomenon observed was the development of petechiæ on the lower extremities in 2 cases. These disappeared in the course of a few weeks as the platelet counts gradually rose.

One patient (Case 3) is of particular interest because he died with the blood picture of acute leukopenic myelogenous leukemia after having a remission of 13 months. This patient was a 63 year old man who had had symptoms attributable to the polycythemia for 3½ years prior to the administration of radioactive phosphorus. He received 1 injection of 7 mc. 18 months before death occurred. Thirteen months after administration of radioactive phosphorus, his family physician had found that the erythrocyte count was high and had withdrawn 1000 cc. of blood. Six weeks later the patient complained of blurring of vision and a sensation of pressure in his head, and returned to the clinic for reëxamination. The values for the hemoglobin, the erythrocyte count and the hematocrit reading were normal; the leukocyte count was 4400 per c.mm. and examination of the blood smears revealed no abnormalities aside from signs of a slight increase in regeneration of erythrocytes. He was sent home, but returned 2 months later, only 2 months before death, complaining of marked weakness and dyspnea on exertion. One week previously he had noted "tarry" stools and had continued to pass black stools for 3 days. Examination disclosed marked pallor and a few scattered petechiæ on the lower limbs. The axillary lymph nodes were moderately enlarged; the spleen and liver were approximately the same size as they had been at the time of his previous visits to the clinic. The value for the hemoglobin was 5.5 gm. per 100 cc. of blood, the erythrocyte count was 2,010,000, the leukocyte count was 1600 and platelet count was 40,000. Examination of blood smears showed abundant myeloid immaturity. Myeloblasts were comparatively numerous, many containing Auer bodies. It was concluded that an acute leukopenic myelogenous leukemia had developed as a terminal event in a case of long-standing polycythemia vera.

The fact has long been recognized that polycythemia vera may terminate with the clinical and hematologic picture of chronic myelogenous leukemia; but the development of an acute leukemia terminally is unusual. From a theoretical viewpoint the possibility of a causal relationship between the administration of radioactive phosphorus and the occurrence of acute leukemia should be given consideration, but

such a relationship does not seem likely for 2 reasons: (1) the first definite signs of leukemia developed long after the material had lost its radioactivity except for an infinitesimally small quantity, and (2) signs of leukemia did not appear in other patients receiving treatment over considerably longer periods of time.

Comment. The results in this series of cases compare favorably with those in the literature. Low-Beer, Lawrence and Stone[4] observed partial or complete remissions in 11 of 14 cases of polycythemia vera; the remissions lasted from several months to 2 years. Fitz-Hugh and Hodes[2] noted marked improvement in 4 of 8 cases, slight improvement in 1 case, but no improvement in 1. In the 2 remaining cases the patients had not been treated a sufficient length of time to warrant comment. Erf and Jones[1] reported satisfactory remissions in 11 cases, and Hemplemann and his associates[3] in a preliminary report on the hematologic complications of radioactive phosphorus therapy, stated that they had treated 7 patients with this disease, but the results obtained were not discussed in the paper.

Excluding Hemplemann's cases because the details of treatment are not yet available, the effect of treatment with radioactive phosphorus has been reported in 33 cases. This number together with the 12 cases which we have reported, totals 45 cases in which radioactive phosphorus has been employed. Partial or complete remissions were observed in 37 of the 45 cases. Of the 8 remaining cases, 2 had not been observed a sufficient length of time to warrant comment, in 2 cases the therapy had not been adequate, and in 4 cases the patients were not improved.

On the basis of the reports in the literature and our own experience, it is our impression that the effectiveness of radioactive phosphorus in the treatment of polycythemia vera is well established. Patients obtaining satisfactory remissions not only obtain complete relief of symptoms attributable to the polycythemia vera, but they do not require any other type of therapy during the period of the remission. On the other hand, in cases in which partial or incomplete remissions occur, some other type of therapy may be required to control the disease satisfactorily, at least until an adequate supply of radioactive phosphorus is available. We would like to emphasize that the dose of radioactive phosphorus must be individualized for each patient. Patients with a mild form of the disease require much smaller dosages than do those with severe polycythemia vera. Periodic examinations of the blood are essential throughout the period of the remission, and with recurrence of the polycythemia, additional radioactive phosphorus should be given.

It is thought that the action of radioactive phosphorus is similar to that of Roentgen therapy of the bone marrow, both types of irradiation resulting in destruction of the cells. Marshak[5,6] has presented evidence indicating that Roentgen irradiation decreases erythropoiesis by retarding mitosis of normoblasts in early prophase. In any event, it seems more logical to employ an agent which acts directly on the bone marrow by diminishing erythropoiesis than to utilize methods which stimulate the marrow to form more cells, as phenylhydrazine

therapy and venesection both do. Although complications in the form of leukopenia, thrombocytopenia or anemia may occur with internal radiation therapy, serious damage to the bone marrow can be averted by frequent and careful hematologic studies. The advantages of this type of therapy seem to outweigh the slight risk involved.

Conclusions. In conclusion, on the basis of reports of other authors and our own experience, we feel that the effectiveness of radioactive phosphorus as a therapeutic agent in polycythemia vera is well established. This type of treatment is in no way curative; but the disease can be more readily controlled with internal radiation than with any other therapeutic procedure now available. Remissions lasting from 9 to 26 months were observed in our cases. The results obtained, the ease of administration, the absence of radiation sickness and toxic symptoms, the concentrated effect of the irradiation on the cells of the bone marrow, together with the fact that radioactivity disappears gradually and dosage can be adequately controlled indicate that this method of treatment is superior to other methods employed in the past. However, leukopenia, thrombocytopenia and anemia may be produced and adequate observation is essential to prevent irreversible damage to the bone marrow.

It is good to know that an adequate supply of radioactive phosphorus will be available when the necessities of war have passed. It will then be possible to determine accurately the proper frequency of administration and effective dosage. Although it has been demonstrated that intravenous administration is preferable for various reasons, yet because of the desirability of oral administration from a practical standpoint, this method of administration should be carefully evaluated.

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RECENT STUDIES ON YELLOW BONE MARROW EXTRACTS

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In 1933 C. W. Watkins[4] of the Mayo Clinic announced that beneficial results could be obtained in the treatment of agranulocytosis by the

oral administration of strained yellow bone marrow. This was confirmed by M. J. Flipse,[1] and later C. M. Marberg and H. O. Wiles[2] demonstrated that the active leukopoietic principle was present in the unsaponifiable fraction of yellow bone marrow. This fraction was employed in the work to be described. The purpose of this investigation was to develop a suitable assay method which would be specific for yellow bone marrow concentrate.

Materials and Methods. The rabbit was chosen as the test animal because of greater constancy in his total and differential white blood cell counts than in those of guinea pigs and rats in our colonies. The average white blood cell count was 11,200 (range, 7900 to 13,300). The average percentage of segmented neutrophils was 31% (range, 27 to 42%). In order to determine the daily and hourly fluctuation of the total white and differential blood cell counts, estimations were made at 2 hour intervals for 3 consecutive days prior to the actual assay. None of the animals studied exhibited marked fluctuation.

It was discovered that the intramuscular injection of small quantities of the unsaponifiable fraction of yellow bone marrow produced a sharp increase in the number of segmented neutrophils in the circulating blood. Control experiments were conducted using comparable doses of olive oil, cod liver oil, carotene in oil and parenteral liver extracts. Each substance effected increases comparable to those observed after yellow bone marrow administration. The effect, therefore, was non-specific.

It was apparent from these results that it might be necessary to simulate human agranulocytosis in experimental animals in order to provide a test object. Several of the techniques described in the literature were investigated. One of the most convenient was a modification of a procedure for poisoning rabbits with benzene described by Kracke.[2]

Each of 10 rabbits was given 1 cc. daily subcutaneous injections of a solution consisting of 1 part benzene to 1 part olive oil for 30 consecutive days. A mild granulocytic leukocytosis rather than a leukopenia resulted. It was found, however, that by increasing the dosage to 2 daily subcutaneous injections of a solution containing 5 parts of benzene to 1 part of olive oil, it was possible to deplete 80% of the rabbits to a level of 2500 white blood cells per c.mm. in an average of 10 days.

Blood Picture of Benzene Treated Rabbits. A primary rise in the total white blood cell count of 2000 to 5000 cells per c.mm. was observed during the first few days of benzene administration. This increase was followed by a cyclic fluctuation in periods of 2 to 4 days. The peaks became progressively lower until total leukocyte counts of 2500 cells per c.mm. or less persisted.

Myeloid Cells. Wright's staining method was used. There was an initial rise in the absolute number and the relative percentage of myeloid cells. This was followed by a cyclic fluctuation which showed a progressive shift to the left in Schilling's index. Toxic characteristics, consisting of large forms with pale staining, irregular shaped nuclei and large amounts of "muddy," bluish staining cytoplasm, were noticed after 5 or 6 days of benzene administration. The picture became progressively worse until a relative myeloid percentage of 10% or less was found.

Lymphoid Cells. Here again an initial increase in the absolute number of lymphocytes occurred during the first few days of benzene administration, followed by a cyclic fluctuation in which the relative

percentage was increased after each rise. Large cells characterized by considerable amounts of bluish cytoplasm and azure cytoplasmic granules were observed.

Monocytes. A slight increase in monocytes was found in the majority of rabbits under test.

Erythrocytes. A mild hyperchromic, macrocytic type of anemia became evident after 10 days of benzene therapy. Rabbits with prolonged depletion times, however, exhibited a more severe idiopathic-hypochromic type. This was, in part at least, due to a general nutritional deficiency since these rabbits usually refused food and lost considerable weight.

Thrombocytes. Thrombocytes were reduced from a starting average of 280,000 to 160,000 per c.mm.

Coagulation Time. The average coagulation time of the blood was increased from 4 to 8 minutes.

Body Temperature. The body temperature of rabbits receiving benzene was elevated from 103° F. to an average of 106° F.

Physical Appearance. All the test animals displayed symptoms characterized by weakness, distress, incoördination and dry coats. The average loss of weight during the depletion period was 200 gm. per rabbit.

Bone Marrow (Femur). Bone marrow samples were taken aseptically before and after benzene treatment, and at the end of the test. The rabbits were given a light ether anesthesia and a 1 inch horizontal incision made along the upper one-third of the femur. A small puncture was then made into the bone marrow cavity with an electric dental drill and a desired amount of bone marrow removed by aspiration. Smears were made by the "imprint" method and stained with Wright's stain. The smears were examined and designated as aplastic or hyperplastic as compared to reference photomicrographs shown below.

Photomicrographs were taken from preparations selected as being representative of the various phases in depletion and repletion as determined in previous experiments.

Response to Therapy of Benzene Depleted Rabbits. A series of experiments were carried out on benzene depleted rabbits to determine the leukopoietic value of yellow bone marrow extracts compared with that of other materials tested on normal rabbits. The results are shown in Table 1. Erythrocytes and hemoglobin were not affected significantly and are not shown here.

The rabbits used in these experiments were male albino rabbits weighing approximately 2.5 kg. These rabbits were depleted by means of twice daily subcutaneous injection of a 5:1 benzene olive oil solution. Benzene was discontinued when leukocyte counts of 2500 cells per c.mm. persisted for 2 consecutive days and daily intramuscular injections of test material begun. Maximum response was obtained from a daily dose of material representing 21 mg. of the unsaponifiable fraction of yellow bone marrow. Smaller amounts gave inconsistent results.

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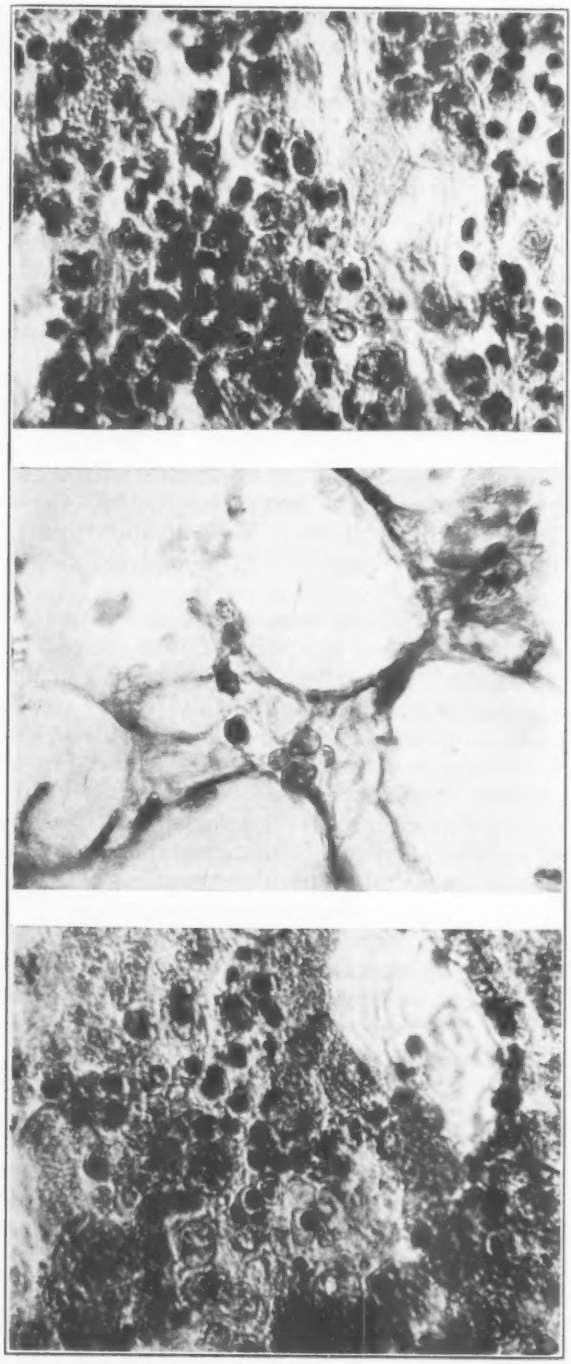

FIG. 1.—Normoplastic control, normal rabbit bone marrow (femur).

FIG. 2.—Aplastic control (femur), benzene treated, olive oil treated.

FIG. 3.—Hyperplastic control (femur), benzene depleted, yellow bone marrow treated.

TABLE 1.—LEUKOCYTE COUNTS IN DEPLETED RABBITS BEFORE AND AFTER TREATMENT

		GROUP A	GROUP B	GROUP C	GROUP D	GROUP E
Therapy (½ cc. daily for 14 days)		Y.B.M. in olive oil	Olive oil	Cod liver oil	15 units parenteral liver	Carotene concentrate
Number of rabbits depleted		27	27	3	3	6
Total white cells per c.mm. at start of therapy	Mean	**2,331**	**2,044**	**1,933**	**2,133**	**2,516**
	Stand. dev.	477	543	511	603	471
	S. E. mean	±93.6	±106.7	±361	±426	±208
Total segmented neutrophils at start of therapy (per c.mm.)	Mean	**378**	**249**	**228**	**410**	**278**
	Stand. dev.	492	274	658	154	195
	S. E. mean	±96.6	±53.8	±465	±108.9	±86.2
Number of rabbits surviving therapy		26	22	3	2	5
Average weight change during therapy		+192	+20	+220	+57	−10
Condition of bone marrow at end of therapy		Hyperplastic	Aplastic	Aplastic	Aplastic	Aplastic
Total white cells per c.mm. at end of therapy	Mean	**11,403**	**3,376**	**3,017**	**2,550**	**5,240**
	Stand. dev.	3,868	1,933	451	636	1,694
	S. E. mean	±773	±422	±319	±636	±847
Total segmented neutrophils per c.mm. at end of therapy	Mean	**4,473**	**690**	**327**	**192**	**723**
	Stand. dev.	1,863	855	319	153	638
	S. E. mean	±332.6	±186.5	±225	±153	±319

Summary. Normal, untreated, albino rabbits were given comparable amounts of 15 unit liver extract (Armour), cod liver oil, whole milk, concentrated carotene solutions and extracts of yellow bone marrow by intramuscular injection. There was a comparable increase in the absolute number and relative percentage of circulating granulocytes, within 4 to 6 hours after administration of test material. The response in each case was similar and therefore non-specific.

Following a modification of the procedure described by Kracke[2] of giving twice daily 1 cc. subcutaneous doses of a solution consisting of 5 parts of benzene to 1 part of olive oil, approximately 80% of the rabbits developed a granulocytopenia in an average period of 10 days.

Rabbits which were benzene poisoned in this manner and given daily intramuscular doses of Armour's 15 unit liver extract, cod liver oil, whole milk, or concentrated carotene solutions for 14 consecutive days showed no evidence of either physical, leukocytic or clinical response. Similarly poisoned rabbits, however, when given comparable doses of yellow bone marrow extracts showed immediate response and complete physical, leukocytic and clinical recovery by the 14th day.

Conclusion. Extracts of yellow bone marrow, in contrast to all other materials tested, contain a specific substance or substances which stimulate leukopoiesis in benzene poisoned rabbits.

The authors are indebted to D. V. Hall for the statistical treatment of the results obtained.

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XUM

ABDOMINAL CRISES IN UNCOMPLICATED SICKLE CELL ANEMIA

A Clinico-pathologic Study of 11 Cases With a Suggested Explanation of Their Cause

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The cause of the so-called abdominal crises in sickle cell anemia is unknown. There is also a general impression that death as a result of uncomplicated sickle cell anemia is relatively rare. Steinberg[1] in a review of sickle cell anemia states "The prevailing conception is that sickle cell anemia *per se* is not fatal." Wintrobe[2] leaves the same general impression in discussing the complications and prognosis in sickle cell anemia. Some of the cases of sickle cell anemia in this institution reveal a different picture and a clinico-pathologic study of abdominal crises in uncomplicated sickle cell anemia with a physiologic explanation of the crises and cause of death is presented.

Material. The autopsy protocols of this institution have been studied and the racial incidence of sicklemia and sickle cell anemia in this area is given in another report.[3] In these protocols there are 24 cases in which a diagnosis of sickle cell anemia was made following postmortem examination. Of these, 13 cases were accompanied by other conditions which make it highly probable that sickle cell anemia *per se* was not the important factor in their deaths. The pertinent autopsy findings and causes of death in these 13 cases are given in Table 1. A critical review of these cases raises considerable doubt regarding the presence of sickle cell anemia in Cases 10, 11, 12 and 13; they seem to fit more clearly into the group of sicklemia patients dying from varied causes with no clinical or autopsy evidence of sickle cell anemia. In the remaining 9 of these 13 cases there was clinical and autopsy evidence of sickle cell anemia but other conditions were present at autopsy which are more probably the actual causes of death.

The material forming the basis of this report consists of the remaining 11 cases of active sickle cell anemia presenting the picture of abdominal crisis in sickle cell anemia with no complicating chronic illnesses.

Case Reports. In this group of cases that represent abdominal crises in uncomplicated sickle cell anemia there is a striking clinical and autopsy picture which will be given in some detail. All these cases were negative for malaria parasites on both thin and thick smears where the patient was admitted to the hospital and no malaria parasites were found in the blood and tissue smears taken in all cases at autopsy. Blood sera taken during life and in all autopsy specimens were negative to both Kahn and Wassermann examinations. Autopsies were performed on all 11 cases.

Case 1. A Panamanian female child, aged 8 months, was admitted Aug. 18, 1939, because of vomiting, weight loss, and grunting respirations of intermittent character during the past 2 weeks. There had been no previous illnesses. Physical examination revealed a temperature of 100° F. The baby was dehydrated and emaciated, with dry hot skin. The cervical lymph nodes were slightly enlarged but not soft or tender. No changes were demonstrated in the heart. There were a few coarse râles at the bases of both lungs. The

abdomen was tense but not rigid and the liver and spleen were not palpable. Admission hemogram was: hemoglobin 25% (Sahli), erythrocytes 2,130,000 per c.mm., and leukocytes 22,800 per c.mm. Differential was: neutrophils 54 and lymphocytes 46. There were 6 nucleated erythrocytes encountered in counting 100 leukocytes and the erythrocytes showed 5% sickled forms on the smear. Urinalysis was negative.

TABLE 1.—AUTOPSY FINDINGS IN 13 OTHER CASES DIAGNOSED AS SICKLE CELL ANEMIA WITH DEATH RESULTING FROM OTHER CONDITIONS

Case No.	1	2	3	4	5	6	7	8	9	10	11	12	13
Race*	BWI	Pan	BWI	BWI	Pan	BWI	Pan	BWI Pan	BWI	BWI Pan	BWI	Pan	Pan
Sex	M	M	F	M	M	F	F	F	F	F	F	M	M
Age (yrs.)	5/12	1	1½	3	7	11	22	22	23	23	36	43	51
Leg ulcers, healed	..	..	..	+	..	..	+	+					
Peripheral edema	+	..	..	..	..	..	+	..	..	..	+	+	
Marasmus	+	+											
Jaundice	+	..	..	..	..	+	..	..	..	..	..	..	+
Rickets	..	..	..	+									
Mastoiditis, purulent	..	..	..	..	+								
Purulent meningitis	..	..	..	..	+								
Brain:													
Edema	..	..	..	..	..	..	..	..	..	+	..	+	+
Abscess	..	..	..	..	..	+							
Arteriosclerosis	..	..	..	..	..	..	..	..	..	..	..	+	+
Lung:													
Chronic passive con.	..	..	..	+	..	+	+	..	+	..	+	+	+
Bronchopneumonia	+	+	..	+	+	+	..	..	..	..	..	+	+
Infarction	..	..	..	..	..	..	+						
Vessel thrombosis	..	..	..	..	..	..	+						
Pleural effusion	+	+	..	..	..	..	..	..	..	+	+	+	+
Heart:													
Hypertrophy	..	..	..	..	..	+	+	..	..	..	+	..	+
Endocarditis	..	..	..	..	..	..	+						
Pericardial effusion	..	+	..	..	..	..	..	..	..	..	+		
Ascites	..	..	..	..	..	+	+	..	..	+	+	+	+
Liver:													
Chr. pass. congest.	+	..	..	..	..	+	+	..	..	..	+	+	+
Chr. hepatitis	..	..	+	..	..	..	..	+	+	..	+	+	+
Cirrhosis, luetic	+												
Glomerulonephritis:													
Acute	..	..	..	..	..	..	..	..	+				
Chronic	..	..	..	..	+	..	+	..	..	..	+		
Interstitial nephritis	..	..	+	..	..	..	..	..	..	..	..	..	+
Nephrosis	..	..	..	..	..	..	..	..	..	..	..	+	
Pyelitis	..	..	..	..	..	..	..	..	..	+			
Pyosalpingitis	..	..	..	..	..	..	..	..	+				
Cellulitis of arms and legs	..	..	+	..	..	..	..	..	..	..	..	..	+
Postpartum septicemia	..	..	..	..	..	..	..	..	..	+			
Septicemia	..	..	..	..	..	+	..	..	..	..	..	..	+
Liver weight (gm.)	155	265	160	535	800	620	1300	890	2100	1200	1090	1630	2920
Spleen weight (gm.)	20	20	15	50	150	5	190	140	20	420	185	505	700
Cause of death	Congenital lues	Marasmus and bronchopneumonia	Cellulitis of leg with toxic hepatitis	Rickets	Mastoiditis with meningitis	Septicemia with brain abscess	Chronic glomerulonephritis, mural endocarditis	Pyosalpingitis, death during ether anesthesia	Postpartum septicemia	Congenital mobile colon with torsion and gangrene	Chronic glomerulonephritis	Nephrosis	Acute toxic anemia with septicemia

* BWI, British West Indian; Pan, Panamanian.

Clinical Course. The child was quite weak and vomited every other feeding. Hartman's solution was given frequently. The baby gained weight for 5 days and then gradually began to lose, seeming to be a feeding problem. At 4 P.M. on August 31 she became very restless, vomited profusely, and the temperature dropped to 97.4° F., with a rapid pulse and respiratory rate. At 2 A.M., September 1, she was grunting, cyanotic, and the peripheral circulation was

collapsed. She was given supportive treatment (no transfusions) but expired at 3:46 P.M., September 1, approximately 12 hours after the onset of the acute abdominal symptoms.

CASE 2. A Panamanian-Costa Rican male, aged 11 months, was admitted May 30, 1944 at 9:15 A.M., with the following history obtained from the mother. About 2 A.M. the day of admission the baby started to scream, then groan and grunt as though he had pain in his stomach. The eyes rolled about and he had minor rigid moments but no convulsions. His mother noted that the hands were swollen. The baby had passed 2 normal stools that morning, vomited twice, and refused both breast and bottle feedings. There had been no previous illnesses. Physical examination revealed a moribund, dehydrated baby who was breathing irregularly and rolling its eyes as though unconscious. There was no spasticity of the body. It was noted that the hands and feet were edematous, that the mucous membranes were pale, and there were a few petechiæ over the legs. The baby expired during the examination at 9:55 A.M., 40 minutes after admission and approximately 8 hours after the onset of acute symptoms.

CASE 3. A Panamanian girl, aged 19 months, was dead upon arrival of a physician at her home July 7, 1942; death was said to have occurred approximately 10 minutes before. The history as obtained from the child's family was that she developed "a cold" the morning of July 5. In the afternoon she was listless, moaned occasionally, and had severe nausea and vomiting. No blood was seen in the vomitus. The past history revealed a similar episode approximately 2 months before, lasting 1 day, and the child apparently recovered without medical care.

CASE 4. A British West Indian female, aged 2 years 3 months, was admitted March 19, 1930, with fever, jaundice, and pain in the right arm. The fever and pain in the arm began the day before admission; jaundice had been present for 1 week. Past history revealed no illness and apparently normal development. Physical examination revealed temperature 101° F., pulse 140, and respirations 30 per minute. The heart and lungs were negative. The abdomen was distended and tympanitic with the liver palpably enlarged. The spleen could not be palpated. The lower two-thirds of the right arm showed some swelling and tenderness but no localization or fluctuation. Roentgenogram revealed slight periostitis on the inner aspect of the lower two-thirds of the humerus. This cleared up spontaneously in the following 10 days. Admission urinalysis and subsequent ones were within normal limits. No blood chemical studies were recorded. Admission hemogram was: hemoglobin 60% (Sahli), erythrocytes 3,300,000, leukocytes 23,100 per c.mm. (neutrophils 68, eosinophils 2, lymphocytes 30%). There were 2 nucleated erythrocytes encountered in counting 100 leukocytes and approximately 5% of the erythrocytes were sickled.

Clinical Course. The patient's jaundice did not increase clinically. The temperature remained around 99° F. and the patient had no complaints until April 17, 1930, when she suddenly complained of severe abdominal pain, with the onset of nausea and vomiting. Hemograms in the interim had been similar to the one on admission. Hemogram taken after the onset of abdominal pain was: hemoglobin 55% (Sahli), erythrocytes 2,460,000; leukocytes 15,000 per c.mm. (neutrophils 52, lymphocytes 48%). Ten nucleated erythrocytes were encountered, and approximately 15% of the erythrocytes on the smear were sickled. The evening of April 17 the temperature was 103° F., with pulse 150, and respirations 40 per minute. The next day vomiting of dark bile-colored fluid and intense abdominal pain continued. The temperature was 104° F. On April 18 the vomitus became blood-tinged; the abdomen was firm and tympanitic. The temperature was 106° F., with pulse 160, thready, and respirations 60 per minute. Shock and collapse were apparent and the patient was irrational and hyperexcitable. Hemogram April 19 was: hemoglobin 65% (Sahli), erythrocytes 2,840,000 and leukocytes 42,900 per c.mm. (neutrophils 75, lymphocytes 25%). Fifteen nucleated erythrocytes were encountered, and approximately 25% of the erythrocytes were sickled.

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The patient expired in typical circulatory collapse and shock at noon April 19, 1930, approximately 48 hours after the onset of the acute abdominal pain.

CASE 5. A Honduran-Nicaraguan male, aged 3 years 7 months, was admitted the last time May 21, 1944, at 4:30 P.M. with complaints of vomiting and convulsions. The child's illness really began March 14, 1944, with the sudden onset of nausea, vomiting, and lethargy followed in 3 hours by a convulsion. The convulsion was characterized by opisthotonos, clenching of the hands, and rolling of the eyes. Following the convulsions, coma would last about 4 hours and then there was marked disorientation until the next convulsion. The convulsions occurred about every 8 hours, did not increase in severity, and lasted about 45 seconds. They were not precipitated by light, sound, or motion. The past history at this time was negative and there had been no similar attacks. Physical examination at this time revealed a well-developed, well-nourished, anemic, disoriented child with temperature of 102.5° F.; no pulse or respiratory rates were recorded. The skin was hot and dry and there was moderate peripheral edema present. The superficial lymph nodes were just palpable. The heart was not enlarged, there were no murmurs, and the rate was rapid. The lungs were clear. The abdomen was soft and no masses were palpable. The pupils were equal, round, and reacted sluggishly to light. Neurologic examination revealed bilateral hyperactive deep reflexes which were equal in the upper and lower extremities. The abdominal reflex was hypoactive on the left side. Babinski reflexes were negative. Admission hemogram was: hemoglobin 26% (Haden-Hausser), erythrocytes 2,060,000 and leukocytes 44,700 per c.mm. (neutrophils 64, lymphocytes 36%). Eight nucleated erythrocytes were encountered but no notation was made of sickled forms. Urinalysis was negative. Spinal fluid cell counts on 3 successive days following admission were 3, 3 and 5 cells per c.mm.; smears and cultures were negative and no pellicle formation was present. Blood and stool cultures were repeatedly negative.

Clinical Course. The temperature ranged in the vicinity of 105° F. for 3 days following admission in spite of sulfadiazine therapy. On March 18 a transfusion of 300 cc. of citrated blood was given with no reaction and the temperature leveled off and returned to normal in 2 days. The convulsions likewise stopped; there was one the morning following the transfusion. The patient was somewhat disoriented for 6 days following the last convulsion and on March 27 seemed to be mentally clear. At this time hemogram was: hemoglobin 52% (Haden-Hausser), erythrocytes 3,120,000 and leukocytes 9500 per c.mm. Differential count was normal and no nucleated erythrocytes or sickled forms were recorded. Neurologic consultation at this time showed no residual involvement and no diagnosis was made. Patient was kept in the hospital for 3 weeks with no recurrence of signs or symptoms and then discharged to the Pediatric Clinic.

Following discharge the child was apparently well and had been seen in the clinic. On May 19, 1944, he complained of abdominal distress, started vomiting, and refused food. He was taken to the Pediatric Clinic on May 21, and at 3 P.M., while there, had a convulsion identical with those of the previous admission. He was admitted to the hospital at 4 P.M. the same day. His temperature was 99° F.; no pulse or respiratory rates were recorded. No physical examination was done due to the convulsions which were severe, lasted approximately 5 minutes, and occurred about every 4 hours. Admission hemogram was: hemoglobin 25% (Tallqvist), erythrocytes 1,900,000 and leukocytes 47,500 per c.mm. (neutrophils 40, eosinophils 5, lymphocytes 55%). Five nucleated erythrocytes were encountered but no sickled forms were mentioned. The clinical course was downhill with repeated convulsions. The day following admission he developed râles in both bases, and the temperature was 105° F., with pulse 170 and respirations 56 per minute. The last convulsion was at 5:30 P.M., May 22, and the patient was comatose following this until death, May 23, 1944, at 1:30 A.M., approximately 33 hours after admission to the hospital.

CASE 6. A British West Indian-Panamanian male, aged 4 years 1 month, was admitted April 4, 1935, at 7:10 A.M. with complaints of stiffness of neck and stupor. The boy had seemed well until the night before admission when he cried, had stiffness of the extremities, and would not respond to questions. The past medical history revealed 2 single convulsive seizures similar to this attack in the past year; no further information concerning these could be obtained. The statement was made that he had never been a strong child. Physical examination revealed a seriously ill, comatose, poorly developed child with temperature of 97.6° F., pulse 158, and respiratory rate 56 per minute. There was a small ulcer along the lateral malleolus of the left leg. The mucous membranes and conjunctivæ were pale. There was a faint systolic murmur at the apex which was not transmitted. There were coarse râles at the bases of both lungs but no consolidation was present. The abdomen was soft; the spleen was enlarged to the umbilicus; the liver was not palpably enlarged. At the time of examination there was no rigidity of the neck or extremities. The knee jerks were exaggerated and there was bilateral ankle clonus. Bicep, abdominal, and plantar reflexes could not be obtained. Admission hemogram was: hemoglobin 15% (Tallqvist), erythrocytes 590,000 and leukocytes 83,600 per c.mm. (neutrophils 46, lymphocytes 50, monocytes 4%). Many nucleated erythrocytes and sickled forms were seen in the smear. No urine was obtained. Spinal fluid was clear, under no increased pressure, and no pellicle formed on standing; smears and cultures were negative. Icterus index was 10 units. The patient expired at 10:10 A.M., April 4, 1935, 3 hours after admission.

CASE 7. A British West Indian female, aged 4 years 7 months, was admitted September 10, 1933, at 11:30 A.M. She was brought to the hospital by a family friend who left immediately. The child expired 10 minutes later and was not seen by a physician. The father was located and gave the following information. The child had developed fever the previous afternoon and in the evening cried with pain in the abdomen. The morning of admission she was restless, irrational, and vomited several times. The family friend called a physician who immediately upon seeing the child ordered her to be taken to the hospital.

The past medical history revealed an admission to the hospital March 17, 1932, with complaints of fever, vomiting, abdominal pain and rigidity of 1 day's duration. There had been no prior episodes similar to this and no other illnesses. Physical examination on the previous admission showed temperature of 102° F., pulse 120, and respiratory rate 34 per minute. There was a low grade jaundice present. The mucosal surfaces were pale. The heart and lungs were normal. The abdomen was rigid, tender and distended. The liver was enlarged, extending 3 cm. below the costal border. The spleen extended to the level of the umbilicus and was firm and nodular. Admission hemogram was: hemoglobin 40% (Sahli); erythrocytes 1,700,000; leukocytes 16,000 per c.mm. (neutrophils 35, eosinophils 5, lymphocytes 60%). Many nucleated and sickled forms of erythrocytes were seen on the smear. Urinalyses were positive for urobilin. The clinical course was good with gradual return to normal of the temperature, pulse and respirations. The leukocytosis disappeared in 3 days. The child was given iron and tonics during the two week hospitalization and was discharged by request on March 21, 1932. The discharge hemogram on the earlier hospital stay was: hemoglobin 30% (Sahli), erythrocytes 1,300,000 and leukocytes 7500 per c.mm. The patient had not been seen in the interim before the last admission.

CASE 8. A British West Indian female, aged 5 years 3 months, was admitted January 14, 1931, at 2:55 A.M., with pain in the abdomen, nausea, vomiting and fever. The abdominal pain began on January 11 and vomiting started the next day; fever was first noticed then. Her past history was noncontributory other than the statement "she was subject to colds." Physical examination revealed temperature 98° F., pulse 150, and respiratory rate 64 per minute. The notation was made that the child was obviously in shock and having severe pain. There was marked pallor of the mucous membranes.

The heart was rapid but no murmurs were heard; the pulse was weak. The lungs were clear. The abdomen was distended and rigid, especially in the upper portion. The liver was palpable and firm with the lower edge extending to the umbilicus. The spleen was palpable 2 inches below the anterior costal margin. The superficial lymph nodes were palpably enlarged but not tender.

Clinical Course. An emergency blood count revealed leukocytes 63,000 per c.mm. (neutrophils 42, lymphocytes 48, undetermined cells 10%). There were 30 nucleated erythrocytes encountered and 15% of the erythrocytes were sickled. No hemoglobin or erythrocyte determinations were done and no urine was voided. The patient failed rapidly, became pulseless at 6:30 A.M., and expired at 7 A.M. the same day, 4 hours after admission.

CASE 9. A British West Indian female, aged 9 years 9 months, was found dead at her home upon the arrival of an ambulance to take her to the hospital on December 22, 1940, at 3:30 P.M. The following history was obtained from the parents. The afternoon of December 18 she complained of pain in the upper abdomen which would last for 2 to 3 hours and then disappear for periods of 4 to 5 hours. On December 20 she was very listless, had intermittent pain in the abdomen and a fever. At noon December 22 she was taken to a doctor who told the mother the girl was very sick and sent her home to go to the hospital. On reaching her house she lay down for approximately 15 minutes, had a general convulsion, and died immediately following the convulsion at approximately 3:30 P.M., Dec. 22, 1940, 4 days after the onset of symptoms.

The past medical history revealed an admission to the hospital in December, 1931, for lobar pneumonia; there were no other illnesses. Physical examination at that time was negative beyond a marked anemia and the typical findings of lobar pneumonia. Hemogram was: hemoglobin 29% (Sahli), erythrocytes 1,590,000 and leukocytes 30,000 per c.mm. (neutrophils 88, lymphocytes 11, monocytes 1%). No notation of sickled erythrocytes was made. The patient was taken home by signing a release 5 days after admission and had not been seen in the intervening 9 years.

CASE 10. A British West Indian female, aged 11 years 6 months, was admitted Feb. 17, 1931, at 9 A.M. with complaints of fever, malaise, pain in abdomen, nausea and vomiting. The illness began February 14 with nausea and vomiting, fever, but no chills. This was accompanied by pain in the bones around the knees and in the upper abdomen. The pain had become progressively more severe. On February 16 she had a dose of epsom salts in the morning. That evening the urine was smoky and scant. Past history showed no prior hospitalizations and general good health beyond occasional head colds. Physical examination revealed a normally developed, dehydrated female in severe shock. The skin was hot and dry and there was marked pallor of the mucous membranes. The scleras were jaundiced. The heart and lungs were negative. Temperature was 102° F., pulse 120, and the respiratory rate was 30 per minute. The abdomen was rigid, distended and painful. The liver was enlarged, painful and extended 6 cm. below the costal border. The spleen was not palpable. There was vague tenderness on deep percussion in the costovertebral angles. Admission hemogram was: hemoglobin 45% (Sahli), erythrocytes 1,810,000 and leukocytes 49,400 per c.mm. (neutrophils 52, eosinophils 1, lymphocytes 40, monocytes 7%). There were 116 nucleated erythrocytes encountered in counting 100 leukocytes and the erythrocytes showed a high percentage of sickled forms. Urinalysis was clear, negative for blood, and showed 1+ albumin. Blood culture taken on admission was negative. Icterus index was 39 units.

Clinical Course. The patient failed rapidly, becoming irrational. The temperature went to 104° F. and remained at that level until death. Pulse ranged around 150 and was weak and thread-like. Respirations became more labored and shallow, and reached 64 per minute. She gradually developed râles and pulmonary edema, expiring at 2 P.M., Feb. 18, 1931, 29 hours after admission.

CASE 11. A Panamanian female, aged 18 years 10 months, was admitted Dec. 11, 1931, at 11:20 A.M. in an irrational and acutely ill condition. Accord-

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ing to an accompanying friend the patient was taken suddenly with pain in the adomen, fever, nausea and vomiting the afternoon of December 9. The vomitus became blood-flecked the next day and the patient developed some diarrhea. The morning of admission she was very ill and acted "queer" at times, finally becoming irrational just before admission. Her past history was non-contributory and there had been no similar episodes. Physical examination revealed temperature 103° F., pulse 140, and respiratory rate 48 per minute. She was irrational, apprehensive, and acutely ill. The skin was dry and hot; the mucosal surfaces were pale and anemic. The scleras were jaundiced; the pupils were widely dilated and reacted sluggishly to light.

TABLE 2.—GROSS AUTOPSY FINDINGS IN 11 CASES OF ABDOMINAL CRISIS IN SICKLE CELL ANEMIA

Case No.	1	2	3	4	5	6	7	8	9	10	11
Age in years	0.66	0.9	1.5	2.25	3.65	4.1	4.55	5.25	9.7	11.5	18.7
Jaundice	..	+	..	+	+	..	+	..	+	+	+
Peripheral edema	+	..	+	..	+	+	..	+	..	+	
Leg ulcers, healed	..	..	..	..	+	..	..	+	+	..	+
Leg ulcers, active	..	..	..	..	..	+	+	+			
Palpable lymph nodes	..	+	+	+	+	+	..	..	+		
Brain:											
Edema	..	+	+	+	+	+	+	+	+	+	
Congestion, marked	+	+	+	+	+	+	+	+	+	+	
Petechial hemorrhages	..	..	..	..	+	..	..	+	..	+	
Heart:											
Fatty changes	+	..	..	+	+	+	+	+	..	+	+
Hypertrophy	..	..	..	+	+	+	+	+	+	+	+
Pleural effusion	..	+	+	..	+	+	+	+			
Lungs:											
Congestion	+	+	..	+	+	..	+	..	+	+	+
Edema	+	+	..	+	+	..	+	..	+	+	+
Ascites	+	+	..	..	..	..	..	+			
Liver:											
Fatty changes	+	+	..	+	+	+	+	+	..	+	+
Congestion	+	+	+	+	+	+	+	+	+	+	+
Petechial hemorrhages	..	..	..	..	+	+	+	..	..	+	
Gallstones	..	..	..	..	..	..	..	..	..	..	+
Spleen:											
Perisplenitis	..	..	..	+	..	+	..	+	+	+	+
Congestion	+	+	+	+	+	+	+	+	+	+	+
Fibrosis	+	+	+	+	+	+	+	+	+	+	+
Infarction	..	..	..	..	..	..	..	..	..	+	
Calcium	..	..	..	..	..	+					
Kidneys:											
Swollen	..	+	+	+	+	+	+	+	..	+	
Scarring	..	..	..	..	..	..	+	..	..	+	
Gastro-intestinal tract:											
Dilated, atonic	+	..	..	..	+	..	+	+			
Congested mucosa	+	+	+	..	+	..	+	+	+		
Petechial hemorrhages	..	..	..	..	+	..	..	+	+		
Lymph nodes, internal:											
Enlarged	+	+	+	+	+	+	+	+	+	+	+
Soft	+	+	+	+	+	+	+	+	+	+	+
Hemorrhagic	..	..	..	..	+						
Bone marrow:											
Hyperplastic	..	+	..	+	+	+	+	+	+	+	+
Soft	..	+	..	..	+	+					
Not mentioned	+	..	+								

The heart sounds were faint, rapid, and there was a weak systolic murmur heard over the apex. The breathing was stertorous and no alveolar sounds could be heard. The abdomen was rigid, distended, and obviously painful on manipulation, especially in the epigastrium. The liver could not be palpated due to the rigidity. The spleen was palpable 3 cm. below the lateral costal border. Hemogram on admission was: hemoglobin 11% (Sahli), erythrocytes 690,000 and leukocytes 30,300 per c.mm. (neutrophils 65, lymphocytes 27, normoblast 8%). No mention was made of sickled erythrocytes. Urinalysis was smoky, positive for hyaline casts and pus cells, and showed 1+ albumin. Icterus index was 38 units.

Clinical Course. The patient failed rapidly. Note was made that attempts to obtain blood specimens for chemistry were unsuccessful due to the complete venous collapse. She expired at 2:30 P.M., Dec. 11, 1931, 3½ hours after entering the hospital.

Autopsy Reports. The autopsy data in the 11 cases are presented in tabular form for the most part, the case numbers corresponding to the cases above. The gross autopsy findings are given in Table 2, the organ weights in Table 3, and the autopsy blood chemistry determinations are presented in Table 4. The microscopic findings are presented in Table 5. The autopsy findings that are due to the sickle cell anemia are discussed in more detail where they vary from other reports and because of the uncomplicated pictures presented in such an early age group.

TABLE 3.—NORMAL* AND AUTOPSY ORGAN WEIGHTS IN 11 CASES OF ABDOMINAL CRISIS IN SICKLE CELL ANEMIA

Case No.	1	2	3	4	5	6	7	8	9	10	11
Sex	F	M	F	F	M	M	F	F	F	F	F
Age in years	0.66	0.9	1.5	2.25	3.65	4.1	4.55	5.25	9.7	11.5	18.7
Body weight (pounds)	8.5	9	16	22	37	28.5	36	40	57	82	101
Body length (inches)	24.5	26	30	33	38	..	42	42	57	59.5	59
Brain:											
Normal weight (gm.)	714	825	1042	1064	1165	1195	1200	1239	1280	1335	1350
Actual weight (gm.)	555	620	850	930	1300	1125	1210	1150	1365	1245	1150
Heart:											
Normal weight (gm.)	37	40	52	56	66	74	80	85	116	124	290
Actual weight (gm.)	35	30	50	80	100	100	132	125	215	275	300
Right lung:											
Normal weight (gm.)	52	59	72	88	90	92	100	107	176	201	350
Actual weight (gm.)	35	40	80	90	200	100	210	160	255	340	245
Left lung:											
Normal weight (gm.)	45	53	65	76	85	89	95	104	160	190	340
Actual weight (gm.)	50	60	50	70	190	75	195	155	230	305	215
Liver:											
Normal weight (gm.)	259	277	345	394	470	523	560	596	825	936	1550
Actual weight (gm.)	175	225	335	650	650	530	635	610	1100	1755	1730
Spleen:											
Normal weight (gm.)	20	26	30	33	39	40	44	47	85	93	150
Actual weight (gm.)	40	25	140	65	200	325	460	150	25	90	1425
Kidneys (combined):											
Normal weight (gm.)	61	71	83	93	106	116	123	129	185	190	260
Actual weight (gm.)	45	60	80	85	140	120	130	150	245	320	220

* Mostly from Coppoletta and Wolbach, Am. J. Path., 9, 55, 1933.

TABLE 4.—AUTOPSY BLOOD CHEMISTRY FINDINGS IN 11 CASES OF ABDOMINAL CRISIS IN SICKLE CELL ANEMIA. (Mgm. per 100 cc.)

Case No.	1	2	4	5	6	8	9	10
Non-protein nitrogen	76.8	36.5	94.4	83.2	42.7	129.0	208.2	64.8
Urea nitrogen	35.1	20.9	61.0	50.5	22.6	84.8	132.4	26.8
Creatinine	1.6	..	1.7	2.5	1.6	4.1	4.0	1.1

Brain. These cases showed extensive degenerative changes in the small capillaries and venules of the brain, particularly in the region of the cortex. There were perivascular fibrosis, edema, infiltration by inflammatory cells, hyaline changes, and occasionally thrombosis or conglutination. There was rather extensive pyramidal cell degeneration with proliferation of fibrous tissue and in 5 cases (ages 1.5, 3.65, 4.55, 9.7 and 18.7 years) there was marked corpora amylacea formation.

Heart. The hearts were hypertrophied as seen in Table 3. There were fatty changes, albuminous degeneration, and edema present, with 3 cases showing perivascular cell infiltrations and degenerative changes in the small vessel walls. Fragmentation of the myocardial fibers was a frequent finding. There were no valvular or endocardial lesions present.

Lungs. In the lungs there were no noteworthy changes of fibrosis or vascular sclerosis, although these have been described as producing cardiac hypertrophy in sickle cell anemia.

TABLE 5.—MICROSCOPIC AUTOPSY FINDINGS IN 11 CASES OF ABDOMINAL CRISIS IN SICKLE CELL ANEMIA

Case No.	1	2	3	4	5	6	7	8	9	10	11
Sex	F	M	F	F	M	M	F	F	F	F	F
Age in years	0.66	0.9	1.5	2.25	3.65	4.1	4.55	5.25	9.7	11.5	18.7
Brain:											
Edema	+	+	+	+	+	+	..	+	+	+	+
Vessel dilatation and engorgement	+	+	+	+	+	+	+	+	+	+	+
Conglutination	+	..	+	+	..	+	..	..	+	+	+
Thrombosis	..	..	..	..	..	..	..	..	..	..	+
Pericapillary fibrosis	+	+	..	..	+	+	..	..	+	..	+
Pericapillary cell infiltration	..	+	+	..	+	+	..	..	+		
Hyaline vessel changes	..	+	+	..	+	..	+	..	+	..	+
Calcosperite formation	..	..	+	..	+	..	+	..	+	..	+
Endothelial cell swelling	..	+	+	+	+	+	..	..	+	..	+
Pyramidal cell degeneration	+	+	+	+	+	+	+	..	+	+	+
Heart:											
Fragmentation	..	..	+	+	+	+	+	+	+	+	
Albuminous degeneration	+	+	+	+	+	+	+	+	..	+	+
Fatty changes	+	..	..	+	..	..	..	+	..	+	+
Edema	+	+	+	+	+	+	..	+	..	+	+
Cell infiltration	..	..	..	..	+	+	..	+			
Lungs:											
Alveolar capillary congestion	+	+	+	+	+	+	+	+	+	..	+
Capillary hemorrhages	..	..	..	+	..	..	+	+			
Alveolar edema	+	+	+	+	+	+	+	+	+	..	+
Bronchopneumonia, early	+	..	+	..	..	..	..	..	..	..	+
Liver:											
Dilatation central veins and sinuses	+	+	+	+	+	+	+	+	+	+	+
Phagocytosis of erythrocytes	+	+	+	+	+	+	+	+	..	+	+
Congestion, general	+	+	+	+	+	+	+	..	+	+	+
Central necrosis	..	..	..	..	..	+	..	+	+	+	
Portal fibrosis	..	..	..	..	..	..	+	+			
Fatty infiltration	+	+	+	+	+	+	+	+	+	+	+
Fatty degeneration	..	..	..	..	..	..	..	+	..	+	
Spleen:											
Fibrosis of capsule and trabeculæ	+	+	+	+	+	+	+	+	+	+	+
"Pooling"	+	+	+	+	+	+	+	+	+	+	+
Phagocytosis of erythrocytes	..	+	..	+	+	..	..	..	..	+	
Hemorrhage and thrombosis	..	..	..	+	..	..	..	..	..	+	
Calcium deposits	..	..	..	+	..	+	..	..	..	+	+
Iron deposits	..	..	..	+	..	+	..	..	..	+	+
Kidneys:											
Vessel congestion	+	+	+	+	+	+	+	+	+	+	+
Interstitial edema	..	..	..	..	..	+	+	+	..	+	
Thrombosed glomeruli	..	..	..	..	..	..	+				
Medullary fibrosis	..	+	+	..	..	..	+				
Granular degeneration of tubules	+	+	+	+	+	+	+	+	..	+	+
Pigment in tubule cells	+	+	+	+	+	+	+	+	+	+	
Calcium in tubule cells	+	..	+	+	+						
Casts	..	..	..	..	..	..	+	..	..	+	
Lymph nodes:											
Hyperplasia and edema	+	+	+	+	+	+	+	+	+	+	+
Phagocytosis of erythrocytes	..	+	+	+	+	+	+	+	+	+	+
Endothelial swelling	..	..	+	+	+	+	..	+			
Gastro-intestinal tract:											
Edema of mucosa	+	+	..	..	+						
Congestion of mucosal capillaries	+	+	..	..	+						
No sections	..	..	+	+	..	+	+	+	+	+	+
Bone marrow:											
Hyperplasia, erythrocytic series	..	+	..	..	+	+	+	+	+	..	..
Phagocytosis of erythrocytes	..	+	..	..	+	..	+				
No sections	+	..	+	+	..	..	..	..	..	+	+

Liver. In the liver no changes were encountered that have not been described before.

Lymph Nodes. The lymph nodes showed marked hyperplasia of the reticuloendothelial cells with extensive phagocytosis of sickled erythrocytes. There was likewise marked edema of the stroma and of the endothelial cells lining the sinuses.

Spleen. The spleens were enlarged and rubbery in 8 of the 11 cases. They all showed marked pooling and congestion with increased fibrous tissue in the capsules, trabeculæ and pulps.

Kidneys. The kidneys showed marked capillary congestion, especially in the glomeruli and the medullary portions. One case showed thrombosis of the glomerular capillaries. There were granular degenerative changes and pigment deposits in the tubules and in 4 cases (ages 0.66, 1.5, 2.25 and 3.65 years) there was calcification of the degenerating tubule cells.

Discussion. CLINICAL PICTURE. A study of these case reports reveals a striking clinical picture of the so-called abdominal crisis in sickle cell anemia, terminating fatally in these instances. It may be summed up as follows: the sudden onset of distress or pain in the abdomen usually accompanied by nausea and vomiting; rigidity or tenderness in the abdomen with occasionally pain or tenderness in the muscles and bones of the extremities not involving the joints; malaise, severe chill followed by fever, most frequently jaundice, early rather rapid peripheral vascular collapse with tachycardia, weak pulse, hyperpnea, and signs of central nervous system involvement. The nervous system symptoms may begin suddenly as a convulsion, usually Jacksonian in character, or may be manifested by symptoms of irrational behavior merging into stupor and coma. Nystagmus was a frequent finding. In Case 6 there was no nausea or vomiting and no abdominal rigidity or tenderness could be found; this patient started his fatal illness with central nervous system symptoms.

At the time these patients were seen the blood showed a severe normochromic anemia with hemoglobin values ranging from 11 to 55% (Sahli). The erythrocytes were usually below 2,000,000 per c.mm.; in Cases 6 and 11 they were below 1,000,000 per c.mm. There were no erythrocyte determinations made in Cases 2, 3, 7, 8 and 9. There was a marked leukocytosis present ranging from 15,000 to 83,500 per c.mm. with a marked increase in the absolute number of mononuclear cells. Nucleated erythrocytes were a constant finding and sickled forms of erythrocytes were noted in 5 of the 7 cases (Cases 1, 4, 6, 8 and 10) in which it was possible to obtain differential smears and probably were present in the 2 cases not so noted. The icterus index was increased where taken, ranging from 10 to 40 units. There was nothing consistent in the urinalyses that were obtained, although only routine chemical examinations were performed.

The clinical picture of these cases is remarkably similar to Wintrobe's[2] description of the symptoms of rapid destruction of blood. He states "Rapid destruction of blood is associated with aching pain in the back, abdomen or limbs, headache, malaise and a severe shaking chill, followed by fever. Profound prostration and shock may occur. The cause of the shock is not clear but one of the important factors in its production may well be a reduction in total blood volume resulting

from the rapid destruction of red cells and their removal from the circulation . . . . It appears that the greatest proportion of the products of red cell destruction is neither left in the circulation or excreted by the kidneys but is rapidly removed by the body tissues."

In the records of these cases different physicians made a diagnosis of shock in 5 of the 7 cases (Cases 1, 4, 8, 10 and 11) in which they were seen alive. In Case 4 there is some evidence of hemoconcentration terminally; in Case 5 it is possible that during the previous admission the patient was relieved of shock by the transfusion which was followed by striking improvement. In none of these cases were blood studies done to determine the presence of hemoconcentration.

The cause of the symptoms outlined above is not felt to be rapid destruction of erythrocytes but the rapid removal of erythrocytes from functional status due to an increase in number of sickled forms, stagnation in capillaries, hemoconcentration and shock as discussed later. Likewise, there is no evidence of intravascular hemolysis in these cases.

Yater and Mollari[4] reported a case of sickle cell anemia in a 25 year old Negress who had been hospitalized 6½ weeks for pain in the legs and 3 weeks before death had a spontaneous miscarriage of a 6 month fetus. Vomiting started 18 hours before death, with severe pain in the region of the liver, marked shock and death. At autopsy small, probably old, infarcts were found in the kidneys and spleen; a questionable thrombus was found in the hepatic artery although no microscopic evidence of liver necrosis was present. The evidence for arterial thrombosis of the liver was doubtful by the authors' own admission and it seems possible that their case may have been one of the abdominal crisis dying in shock similar to those presented above.

In Leivy and Schnabel's[5] report of 3 clinical cases of abdominal crisis in sickle cell anemia, 1 case, a 4 year old Negress, was relieved of the abdominal pain in 3 successive admissions by blood transfusions; the last time a splenectomy was performed. The patient returned on 3 occasions following the operation with the same complaints and was relieved each time by blood transfusions. The spleen removed at operation weighed 200 gm. and showed no evidence of infarction. From a study of the reported cases they felt that the liver and gallbladder could be eliminated as a factor in the production of the symptoms of abdominal crisis. In the first case they reported, a Negro male, aged 29 years, there were marked bony changes in the vertebræ and they suggested root pains as a possible cause for the abdominal crisis although they felt that probably no single explanation would be adequate in all cases.

Campbell,[6] stressing the difficulty of distinguishing between abdominal crises and surgical conditions in the abdomen, reported 6 cases. He states that the most widely accepted theory in explaining the condition is that the pain is caused by splenic infarction or perisplenitis even though there were reports that the pain recurred following splenectomy.

A discussion of the autopsy findings in these cases must be divided into two phases: the changes due to sickle cell anemia and those as a result of the shock present.

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PATHOLOGIC CHANGES DUE TO UNCOMPLICATED SICKLE CELL ANEMIA. *Brain.* In this group of cases the consistent changes were vessel engorgement, perivascular fibrosis and edema, hyaline changes, endothelial swelling and proliferation, marked congestion, small hemorrhages, focal areas of pyramidal cell degeneration with gliosis, and in some cases corpora amylacea formations. There was also conglutination of erythrocytes and early cellular infiltrations around the vessels. In 1 case there was actual vessel thrombosis.

Spinal Cord. In 3 of the 11 cases presented the spinal cords were available for study. There were no degenerative changes found in the cords or in the spinal nerves arising from the cords. The vessels showed some congestion with sickled erythrocytes but no degenerative changes were present in the vessel walls.

Hughes *et al.*[7] reported the autopsy findings in the brains of 2 cases of sickle cell anemia. One case, a 6 year old male, had cerebral symptoms from 20 months of age and died with aphasia, stupor and a residual paralysis from an attack at the age of 3 years. At autopsy there was atrophy of the right cerebral cortex probably due to thrombosis of the right middle cerebral artery, dilatation of the ventricles, chronic adhesive leptomeningitis, acute hemorrhagic leptomeningitis and recent thrombosis of the left middle cerebral artery with early encephalomalacia. The second case, a 26 year old male, had had a "stroke" at the age of 6 years and residual paralysis since then. At autopsy atrophy of the left cerebral cortex, old meningeal and subpial hemorrhages, multiple old subpial cysts, chronic adhesive arachnoiditis, and uremia were found. They felt that the lesions were primarily intravascular with subsequent thrombosis, hemorrhage, and degenerative changes.

Connell[8] reported a case of cerebral necrosis due to "sickle cell disease" in a 20 year old Negress with a history of left-sided paralysis starting 6 years before. At autopsy there was old right cerebral atrophy and also a recent massive left cerebral hemorrhage; histologic study revealed atrophic changes in the pyramidal cells; the vessels showed endothelial cell proliferations, hyaline changes, congestion and thrombosis; there were scattered corpora amylacea throughout the sections.

Bridgers[9] reported 2 cases of cerebral changes in sickle cell anemia. One case, a 62 year old Negress, was in shock and stupor for 3 days before death; autopsy revealed multiple focal necroses and hemorrhages in the brain which were associated with "peculiar hyaline bodies resembling masses of fluid and blood cells within greatly congested capillaries." This case is well beyond the usual accepted age for active sickle cell anemia. The other case, a 4 year old Negress with an old history of paralysis, showed a massive recent hemorrhage with areas of encephalomalacia and some areas of old atrophy. Histologic examination in this case showed old partial occlusion of the larger arteries, fragmentation of the elastic lamina, endothelial proliferations, hyaline vessel changes and some inflammatory cell infiltrations. Case 5 of the series reported here showed the same extreme endothelial proliferations,

old thromboses of medium sized arteries, and focal pyramidal cell degenerations and gliosis. It is interesting that this case had an episode of cerebral symptoms 2 months before, which was terminated clinically by a transfusion. There were no residual symptoms demonstrable; the patient was admitted with the same severe symptoms the last time and expired.

Wertham *et al.*[10] studied 5 cases of sickle cell anemia and give the following conclusions: "The essential neuropathologic features of sickle cell anemia are small necrotic and necrobiotic lesions on a vascular basis, diffusely distributed, with predilection for the border between the cortex and the subcortical white matter; marked general hyperemia and congestion of blood-vessels; hypertrophy and proliferation of endothelial and adventitial elements of the walls of small blood-vessels; siderotic pigment in intra-adventitial spaces and adventitial cells; larger vascular lesions of uncharacteristic type (softenings, thromboses, etc.); small hemorrhages and extravasations; intravascular lipoid material and fat embolism of capillaries and precapillaries; focal and diffuse changes in the nerve cells in cortical and subcortical gray structures, and focal areas of demyelination in the spinal cord, similar to those seen in subacute combined degeneration." Their conclusions are open to question along the following lines: the ages studied were 28, 31, 49, 56 and 65 years; the last 3 are well beyond the usual age of active sickle cell anemia and in none of their cases are any hemograms given. In 2 of the 3 oldest patients a diagnosis was made of arterio- and arteriolosclerosis with hypertension. The patient, aged 31 years, had a ruptured congenital cerebral aneurysm on the left side and a similar unruptured aneurysm on the right side. The lipoid material found in the capillaries in the 2 cases was not explained as to source and they did not demonstrate foci of necrosis in the bone marrow. The spinal cord changes described were found in only 1 case, not identified, and the clinical data given with the cases makes no mention of spinal cord symptoms, abdominal pain, or the other manifestations of crises in sickle cell anemia. Likewise the symptoms of combined degeneration of the spinal cord according to Wechsler[11] are paresthesias, "tabetic" ataxia and rarely pains, although girdle sensations do occur. Wechsler says that in his experience pernicious anemia is the only condition in which combined degeneration occurs, with the possible exception of teniasis.

Wade and Stevenson[12] reported a case of sickle cell anemia in which fat emboli from necrotic foci in the bone marrow produced focal hemorrhage and necrosis in the brain. They, however, mention that Lehman and McNattin found fat emboli in 37 of 50 autopsies and that Vance found it in 7 of 82 cases definitely not associated with trauma or bone marrow necrosis. They felt that in their case of sickle cell anemia the fat emboli were of importance in producing symptoms and the cerebral changes.

Heart. Myocardial hypertrophy was present in 8 of the 11 cases and has been described by Klinefelter.[13] However, he mentions no histologic changes beyond myocardial fiber hypertrophy. Steinberg[1]

mentions frequent observations of patchy fatty degeneration in the myocardium of the cases he reviewed. In these cases muscle fragmentation, fatty changes, scarring, granular degeneration and edema were a constant finding. In 3 cases there were perivascular cell infiltrations; whether these were due to the anoxemia and stagnation of sickle cell anemia or shock or both is difficult to determine. From the relatively advanced changes present it is felt that they may have been a result of the sickle cell anemia. No valvular or endocardial changes were present in any of the cases.

Lung. In these cases there was no histologic evidence of vessel thrombosis, sclerosis or infarction. It is possible that death intervened too rapidly for the production of such changes in these cases; likewise the erythrocytes in the lungs would be obviously better oxygenated than elsewhere and the changes in the vessels due to anoxemia would be longer in starting. Vessel changes, thrombosis and infarction have been described by Yater and Hansmann[14] and Steinberg,[1] although their cases were in the older age groups and had more chronic illnesses in the terminal pictures.

Kidneys. In the kidneys calcification of degenerating tubular epithelial cells has been described in 1 case by Yater and Mollari[4] and was present in 4 of these cases.

Extensive erythrophagocytosis was present in the liver, lymph nodes, spleen and bone marrow of most of these cases. This has been adequately described before, but in view of its probable importance should be stressed again.

Pathologic Changes Due to Non-traumatic Shock. The gross and microscopic findings in shock have been described by Moon.[15] In the 11 cases presented here the gross findings (Table 2) of collapsed peripheral veins, peripheral edema, thick, dark unclotted blood, cerebral congestion and edema, effusions into the body cavities, pulmonary congestion and edema, congestion and edema of the gastro-intestinal tract, and marked congestion of the liver, kidneys and spleen fit his gross picture of shock.

The microscopic changes that are found in shock not complicated by toxemia consist of marked capillary congestion, edema and some petechial hemorrhages with parenchymatous changes in the liver and kidneys if the condition is marked. The heart usually shows only congestion. In these cases (Table 5) the congestion was intense, edema marked, and granular degenerative changes were present in the liver and kidneys. It is felt, as stated before, that the myocardial changes were of considerably longer duration than the shock and probably represent changes due to the sickle cell anemia.

In Table 4 are given the postmortem blood non-protein nitrogen, urea nitrogen and creatinine levels. They are available in 8 of the 11 cases and show nitrogenous retention as Moon describes being present in shock.

The marked leukocytosis found in these cases ranging from 15,000 to 83,600 per c.mm. in the absence of infection clinically or at autopsy is strong evidence for shock. Moon gives a résumé of the literature on this subject.

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MECHANISM OF THE PRODUCTION OF SHOCK. The absence of trauma and infection in the clinical records and at the postmortem examinations of these cases rules out shock from these causes. There is left shock due to a lack of normally functioning erythrocytes. The shock in these cases is obviously not due to hemorrhage and it is not due to intravascular hemolysis because of the constant failure to demonstrate hemoglobinuria in these cases. Haden[16] says that the failure to demonstrate hemoglobinuria is definite evidence that erythrocyte destruction is taking place in the reticulo-endothelial cells of the body and not in the blood stream. Although Sydenstricker[17] reported auto-agglutination *in vitro*, Cardoza[18] showed that the sickling trait was not connected with any one of the isohemagglutinin or immune agglutinogen groups, and he could not isolate any anomalous isohemagglutinins among the cases he studied.

Wiggers[19] states that in the anemic type of anoxia "the oxygen tension is normal but the supply of (oxygen) is deficient owing to a decreased quantity of functional hemoglobin." Best and Taylor[20] discussing this say that the heart rate is increased under these circumstances, thus increasing the rate of blood flow in order that the oxygen delivered in small quantities in anemia may be delivered at the high pressure necessary for tissue oxygenation. Karsner[21] says that anemia produces changes in the myocardial fibers which tend to lessen the blood pressure and thus the blood volume flow in many cases, and certainly in the cases reported here the changes he described were present.

In sickle cell anemia the anemia is constant though somewhat variable in intensity of symptoms. Hahn and Gillespie[22] feel that in the sickled forms of erythrocytes the hemoglobin is in a reduced or uncombined form; if this is true that amount of hemoglobin present in the sickled erythrocytes is of no value for oxygen carbon dioxide carriage in a person already suffering from a lack of hemoglobin. Numerous studies have been published upon the influence of oxygen and carbon dioxide concentrations in sicklemia and sickle cell anemia. *In vitro* studies by Hahn and Gillespie,[22] Scriver and Waugh,[23] and Tomlinson[24] have shown that a reduction of oxygen concentration, an increase of carbon dioxide concentration or the simultaneous change of both produces an increase in the number and degree of sickled erythrocytes. The rapid methods for diagnosing the sickling phenomena by Scriver and Waugh through stagnation of blood in the finger tip and my method of exposing blood to carbon dioxide in a separatory flask are based upon these factors. *In vivo* studies by Klinefelter[13] showed that a reduction of the oxygen content in arterial blood increased the percentage of sickled cells. Using a patient with sickle cell anemia, not in a crisis, the resting control study showed 19% sickled cells in arterial blood; after the administration of 100% oxygen for 20 minutes there were 9.5% sickled cells; after the administration of 10% oxygen for only 10 minutes there were 32% sickled cells in the arterial blood which represents a marked increase in sickled forms for a drop of approximately 10% oxygen concentration from that of nor-

mally inspired atmospheric air. Hahn[25] believes that there is a correlation between the severity of the sickle cell anemia and the number

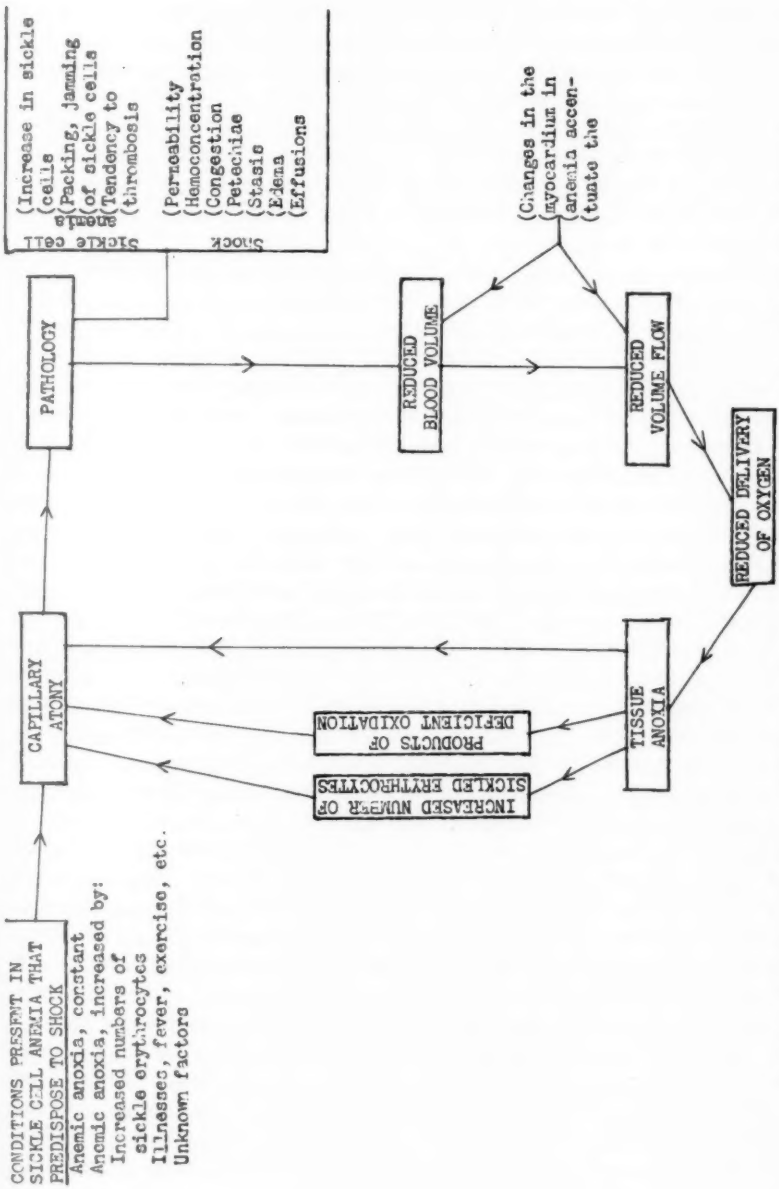

FIG. 1.—Suggested mechanism of the production of shock in sickle cell anemia. (Modified from Moon[15])

of sickled cells in the circulation and Sherman[26] uses the presence of sickled forms in the circulation as a means of differentiating between sickle cell anemia and sicklemia.

It is my belief that under certain conditions, as yet imperfectly known, the anoxia which accompanies active sickle cell anemia probably precipitates a chain of events that frequently results in shock. The conditions that may bring about this change may be the severe anemia (less than 1,000,000 erythrocytes per c.mm. for example), failure of the circulation due to cardiac damage, increased demand upon the oxygen-carrying capacity of the blood by fevers, illnesses, exercise, and so forth, and finally those as yet unknown factors that produce marked erythrocytic destruction in these cases or that increase the number and degree of sickled forms in the circulation and thus lessen the functional hemoglobin present. The mechanism is shown diagrammatically in Figure 1.

With the circulating sickled erythrocytes present in sickle cell anemia we can presume that there are certain degrees of anoxia present in the tissues, especially in the arterial and venous capillaries where the blood is considerably removed from its point of oxygenation.

Krogh[27] and Landis[28] have shown that capillary anoxia results in damage to the lining cells rendering them more permeable to plasma; the escape of plasma produces hemoconcentration which tends to stagnate the blood, slow the circulation through the small vessels and intensify the anoxia while building up the carbon dioxide content which would tend to cause sickling in any susceptible erythrocytes not already showing the change. Diggs and Ching[29] have described as a prominent feature in the microscopic examination of tissues from sickle cell anemia cases "the prominence of capillaries, which are dilated and filled with sickled cells, giving the appearance of packing under pressure. . . . . A possible explanation of the capillary engorgement is that the elongated and spiked cells interlock and pass with more difficulty through narrowed spaces than do normal cells." Considering the possibility of shock being present in these cases, the appearance of packing or jamming may be due to both the hemoconcentration and the abnormal shapes of the erythrocytes. In either event, it is marked in the microscopic examination of sickle cell anemia tissues and the stagnation of blood in the dilated, atonic and damaged capillaries removes considerable numbers of erythrocytes from the circulating blood volume. This tends to accentuate the circulatory deficiency and thus increase the anoxia and intensify the vicious cycle of shock which when continued for a relatively short period of time will terminate fatally with the morphologic changes of shock described by Moon.[15]

There are two other findings in these cases which may play some part in accentuating the shock when it is initiated. Moon[15] has shown that the spleens of dogs in experimental shock, and to a lesser extent in humans, are contracted and relatively bloodless. In these cases of sickle cell anemia there was considerable thickening and fibrosis of the capsules and trabeculæ with scarring of the reticular framework and pulp (presumably the result of previous episodes); such changes would tend to make the spleens less contractile and prevent the accumulation and discharge of any reserve erythrocytes present in the spleens. In spite of the fibrosis, there was often con-

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gestion and pooling in the sections; grossly the spleens were usually enlarged, firm and rubbery. In cases where the spleen was atrophic and small, there would obviously be no reserve of erythrocytes. The other finding is the extensive phagocytosis of sickled erythrocytes by the reticulo-endothelial cells, especially in the liver and lymph nodes, and less marked in the spleen and bone marrow. The exact part this may play is hard to evaluate; however, it does remove some erythrocytes from the deficient and failing circulation. Likewise, the extensive erythrophagocytosis may be one of the factors initiating or participating in the accentuation of the anemia and thus starting the cycle of anoxia that may end in shock.

Comment. In the cases presented here, shock resulting from the anoxia accompanying anemia is felt to play an important part in their symptomatology and deaths. Whether shock enters into the clinical picture of other manifestations of sickle cell anemia will await corroboration by other investigators. The cases of sickle cell anemia seen with acute symptoms by any one person are relatively few in short periods of time; however, if everyone remains aware of the possibility of shock and uses the means available to determine the presence of shock, the validity of the explanation given here will be rapidly checked. Likewise a therapeutic aid in the treatment of severe sickle cell anemia will be available in the use of blood transfusions and plasma to treat the shock. In regard to blood transfusions the sickling of erythrocytes in the usual stored blood from sicklemic individuals and reactions resulting from the use of this blood must be kept in mind, as has been previously described.[24]

Summary. Analysis of 11 cases of abdominal crises in uncomplicated sickle cell anemias showed that 4 cases were dead on arrival at the hospital, or died before they were seen by a physician. Of the 7 cases examined during life notation was made that 5 were in shock. The clinical picture was as follows: sudden onset of distress, pain, rigidity or tenderness in the abdomen usually accompanied by nausea and vomiting, occasional pain or tenderness in muscles and bones, severe malaise, chill, and fever; frequent jaundice, early rapid peripheral vascular collapse with shock and central nervous system involvement. Clinical laboratory studies of these cases revealed severe normochromic anemias (hemoglobin 11 to 55% Sahli), erythrocytes 0.59 to 2.4 million per c.mm.) and marked leukocytosis (15,000 to 83,600 per c.mm.), with an absolute increase in mononuclear cells; nucleated erythrocytes and sickled forms were an almost constant finding and the icterus indices were increased (10 to 40 units).

Postmortem examinations, with special attention to the changes of sickle cell anemia in a young age group with no complicating illnesses, revealed congestive and degenerative changes in the capillaries and small venules of the brain, pyramidal cell degeneration, glial proliferation, and corpora amylacea formations. No spinal cord changes were found beyond vessel congestion. In the hearts there were hypertrophy, fatty changes, albuminous degeneration, edema and fragmentation of the myocardial fibers; 3 instances of vessel degenera-

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tion and perivascular cell infiltrations were present. No endocardial or valvular lesions were found. No changes were found in the lungs beyond marked capillary congestion. The livers showed marked congestion and extensive phagocytosis of sickled erythrocytes by the reticulo-endothelial cells and the same findings were present in the lymph nodes. The spleens were enlarged in 8 of the 11 cases and showed marked congestion and "pooling," together with fibrosis of capsule, trabeculæ and reticular stroma. The kidneys showed marked congestion of the capillaries with glomerular thrombosis in 1 case. The tubules showed granular degenerative changes, pigment deposits, and calcification in 4 cases.

Postmortem examination likewise revealed the following evidence of shock: collapsed peripheral veins, peripheral edema, thick, dark, unclotted blood, cerebral congestion and edema, effusions into the body cavities, pulmonary congestion and edema, congestion and edema of the gastro-intestinal tract with marked congestion of the liver, spleen and kidneys. Autopsy blood chemical determinations showed nitrogen retention. The spleens were enlarged, engorged and fibrosed, which probably prevented them from contracting in shock.

The clinical symptoms at the onset of abdominal crises in sickle cell anemia are remarkably similar to Wintrobe's[2] description of the symptoms of rapid destruction of blood. There is no evidence of extremely rapid blood destruction in these cases; it is felt that the symptoms are due at first to increases in the number of sickled cells and their removal from the circulation with rapidly developing shock producing the terminal symptoms and death.

It is suggested that the mechanism of death in these cases of abdominal crisis in sickle cell anemia is shock. A possible explanation for the shock is: the anoxia accompanying anemias is increased in severity in sickle cell anemia as sickled erythrocytes do not carry or are poor carriers of oxygen to the body tissues; the heart in severe anemia is weakened; sickled erythrocytes have a tendency to pack or jam in small capillaries; the capillary anoxia results in plasma loss, hemoconcentration, stagnation, and the stagnation removes available erythrocytes from the circulation, increasing the circulatory failure, anoxia, and perpetuates the vicious cycle of shock.

Further examination of cases of abdominal crisis and other conditions in sickle cell anemia should be directed toward determining the presence of shock. If shock is present, transfusions and plasma may greatly alter the clinical picture. Transfusions in these cases must not be given from people showing the sickling trait.

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THE ARTIFICIAL PRODUCTION AND SIGNIFICANCE OF TARGET CELLS

WITH SPECIAL REFERENCE TO THEIR OCCURRENCE IN THALASSEMIA (COOLEY'S ERYTHROBLASTIC ANEMIA)

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HADEN and Evans[6] in 1937 first noted in fresh preparations of the blood of patients with sickle cell anemia considerable numbers of erythrocytes which "instead of being biconcave disks had a central 'sugar loaf' elevation so that a cross-section had the appearance of a Mexican hat instead of a dumbbell." Such RBC in stained smears appeared to have a peripheral ring and central dot of hemoglobin separated by an intervening zone of pallor. In some, a bridge of hemoglobin connected the central dot with the peripheral ring. These they referred to as "dimpled corpuscles" and erroneously considered them to occur in significant numbers only in sickle cell anemia.

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In 1938 Barrett,[1] in reporting an extensive series of observations on this type of erythrocyte, introduced the term "target corpuscles," a reference only to their appearance in the stained smear and not to their three-dimensional form. He found this type of erythrocyte with considerable frequency in the blood of patients with biliary obstruction, steatorrhea, certain hypochromic anemias, as well as after splenectomy, and observed large numbers of these cells in jaundiced patients in whom there was no abnormality of the erythrocyte count or hemoglobin value. Experiments were conducted which indicated that the target corpuscles were appreciably more resistant to hemolysis in hypotonic solutions of sodium chloride than were normal erythrocytes. From studies on fresh preparations and cells fixed with formol saline, Barrett concluded that the three-dimensional precursor of the target cell was probably a bowl-shaped corpuscle with a central evagination accounting for the central dot of hemoglobin. Roentgenograms of plasticine models were offered as corroborative evidence that such "umbonate" cells would appear as targets when viewed in transmitted light.

Dameshek[3] confirmed the presence of target cells in conditions other than sickle cell anemia. He and others[8,12] noted their presence in thalassemia major[9] (erythroblastic anemia of Cooley, Mediterranean anemia), and in the milder, genetically related anemia for which the name "thalassemia minor" has recently been proposed.[9] The latter has been shown to be qualitatively similar to the full-blown disease, but quantitatively less severe, and represents the reservoir of genetic carriers who perpetuate the fatal form of the malady. Dameshek[4] referred to anemias of this milder type as "Mediterranean Target-Oval Cell Syndromes," and suggested the possibility that the target cell may be "the inherited factor responsible for the disease known in its full-blown form as Cooley's erythroblastic anemia." Smith[8] has taken exception to this viewpoint, and has concluded that the target cell should be regarded as non-specific.

Bohrod[2] in 1941 suggested that the target cell was merely a young, resistant cell, basing his conclusion on observation of the appearance of target cells in the early days after a brisk hemorrhage, and the presence of target forms among the larger, basophilic, and presumably younger cells seen at this time. He concluded that this type of erythrocyte appeared following blood loss regardless of source, and that these cells might be constantly present if blood loss were constant.

Greenblatt[5] reported in 1943 on the occurrence of a high percentage of target cells in the peripheral blood of a large number of soldiers with acute hepatitis and icterus. During the time target cells were present there was also increased erythrocyte resistance to hypotonic solutions of sodium chloride. As the icterus decreased there was likewise progressive reduction in the number of target corpuscles in the peripheral blood, but no definite correlation between the severity of icterus and the numbers of target cells present could be made.

Because of our particular interest in the target corpuscle in thalassemia major and minor, a group of experiments bearing on the artificial production and nature of the target cell was undertaken.

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Methods. In every experiment, blood was obtained from the antecubital vein in a clean, dry syringe without stasis. Control blood was in every instance placed in a bottle containing a mixture of 40% potassium oxalate and 60% ammonium oxalate. When exactly 5 cc. of blood was added to such containers a 0.2% concentration of the anticoagulant mixture resulted. Heller and Paul[7] have shown that under these conditions there is no alteration in erythrocyte volume. In preliminary experiments, we found that the appearance of the erythrocytes in numerous blood smears made after a short time interval from samples so collected differed in no wise from those in smears made directly from peripheral blood. The containers employed for such control samples will be referred to hereafter as "control hematocrit bottles." All serum and plasma chloride determinations were done by the micro method of Van Slyke.[10] Whenever the volume of packed erythrocytes was determined, a Wintrobe hematocrit tube properly filled with a thoroughly mixed sample of blood was centrifuged for 1 hour at 3000 r.p.m. All smears were made by the coverslip method and stained with Wright's stain.

Presentation of Data. EXP. 1. This experiment was designed to determine whether the erythrocytes of normal individuals can readily be converted to target cells if the blood is rendered hypertonic by the addition of potassium oxalate.

Potassium oxalate bottles were prepared as follows: 1 cc. of 2% potassium oxalate solution was pipetted into thoroughly cleaned bottles and the solution dried so that each bottle contained 0.02 gm. potassium oxalate. In a preliminary experiment, 3, 5 and 7 cc. of blood from a normal individual was placed in such bottles. It was found that the largest percentage of target cells in stained smear was present in blood from the bottle in which 3 cc. had been placed. Accordingly, blood was drawn from 6 normal individuals, and in each instance 3 cc. was added to a potassium oxalate bottle prepared as described above. At the same time 5 cc. of the same blood was added to a control hematocrit bottle. Smears were made from both control and experimental blood and the appearance of the erythrocytes compared. In every case, there were large numbers of target cells present in the hypertonic blood, and only a very scattered few or none in the control.

TABLE 1.—EFFECT OF THE ADDITION OF POTASSIUM OXALATE TO BLOOD ON THE MORPHOLOGY, VOLUME AND RESISTANCE TO HYPOTONIC SALINE OF NORMAL ERYTHROCYTES

Name	Smear	Presence of target cells	Vol. packed RBC	% shrinkage	Hemolysis in hypotonic saline: Begins (%)	Hemolysis in hypotonic saline: Complete (%)
G. B.	Exp. blood	Many	..	..	.48	.30
	Control blood	1 seen	..	..	.48	.30
L. Y.	Exp. blood	Many	..	..	.50	.32
	Control blood	None	..	..	.48	.32
W. G.	Exp. blood	Many	39.8	15.3		
	Control blood	Very rare	47.0			
C. K.	Exp. blood	Many	39.0	15.6		
	Control blood	None	46.2			
W. W.	Exp. blood	Many	37.3	18.6		
	Control blood	None	45.8			
L. F.	Exp. blood	Many	38.5	16.3		
	Control blood	None	46.0			

In some instances, fragility tests were performed on both hypertonic and control blood, while in others the volume of packed erythrocytes

was determined on both samples. Results are summarized in Table 1. The target cells produced in this manner showed no increased resistance to hypotonic solutions of sodium chloride.

EXPERIMENT 2. In this experiment, an attempt was made to determine whether other agents causing hypertonicity were equally effective in producing target cells, or whether the target corpuscles produced in Experiment 1 were the result of some property of potassium oxalate rather than of simple changes in tonicity.

Ten cc. of venous blood was drawn from several normal individuals; 5 cc. was allocated to a control hematocrit bottle. The remaining 5 cc. was added to a second bottle differing from the control container solely in the addition of 15 mg. of dry sodium chloride. Smears were obtained from both samples, and the volume of packed erythrocytes determined as before.

In every case there were large numbers of target cells in the blood to which sodium chloride was added. Results appear in Table 2.

TABLE 2.—EFFECT OF THE ADDITION OF SODIUM CHLORIDE TO BLOOD ON THE MORPHOLOGY AND VOLUME OF NORMAL ERYTHROCYTES

Name	Smear	Presence of target cells	Vol. packed RBC	% shrinkage
W. A.	Exp. blood	Many	41.2	17.3
	Control blood	None	49.8	
F. B.	Exp. blood	Many	37.1	17.0
	Control blood	None	44.7	
A. R.	Exp. blood	Many	37.0	16.1
	Control blood	None	44.1	

EXPERIMENT 3. This experiment was designed to determine whether the erythrocytes of normal individuals can readily be converted to target cells by suspending them in serum rendered hypertonic by simple evaporation and without the addition of chemicals.

TABLE 3.—EFFECT ON MORPHOLOGY OF NORMAL ERYTHROCYTES OF SUSPENDING THEM IN CONCENTRATED SERUM OF SAME PERSON

Name	Smear	Presence of target cells	Serum chlorides (millieq.)
J. V. N.	Exp. blood	Many	148
	Control blood	None	107
W. N. V.	Exp. blood	Many	182
	Control blood	None	103

Twenty-five cc. of venous blood was drawn from 2 normal individuals. The blood was allowed to clot, centrifuged, and the serum pipetted off immediately. Ten cc. samples of the serum thus obtained were allowed to evaporate in a watch glass to roughly 80 and 60% of their original volume respectively. A second 5 cc. sample of blood was then collected from the same persons and placed in a control hematocrit bottle. From this blood, smears were made as controls. The control sample was then centrifuged, the normal plasma pipetted off, and replaced with an equal volume of the concentrated serum from the same individual. The cells were resuspended in the concentrated serum and smears were made. Chloride determinations were done in

each case on the normal plasma and the concentrated serum in order to evaluate the degree of concentration. As before, the hypertonic sample contained large numbers of target cells. The results are shown in Table 3. Photomicrographs of control smears and of smears prepared from cell suspensions in the concentrated serum of the same person are shown in Figure 1.

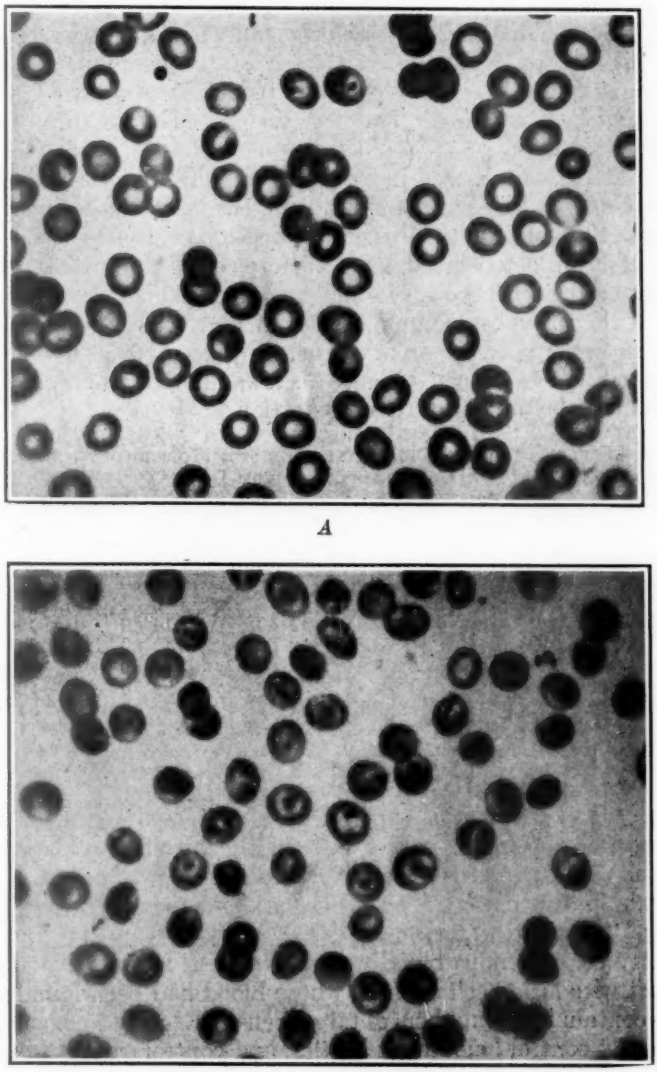


FIG. 1.—*A*, Blood smear of a normal individual (J. V. N., Table 3) showing normal erythrocytes and no target cells. (× 970.) Serum chlorides, 107 milliequivalents.

B, Smear made from the same blood (J. V. N., Table 3) after the centrifuged erythrocytes were resuspended in the hypertonic concentrated serum of the same person. Note the target cells. (× 970.) Serum chlorides, 148 milliequivalents.

EXPERIMENT 4. This experiment was designed to find out if target cells could be produced in the experimental animal as a result of plasma concentration secondary to dehydration.

A 13.65 kg. dog was rapidly dehydrated as follows:* The animal was first placed for 8 hours in a room in which the temperature was maintained around 115° F. He was then removed to a cool room for 16 hours following which he was subjected to another $6\frac{1}{2}$ hours at 115° F. Blood was removed from a leg vein at the start of the experiment, after 8 hours at 115° F., and again when the dog was removed from the hot room at the conclusion of the experiment. At each bleeding, exactly 5 cc. of blood was placed in a control hematocrit bottle and smears made. The dog was denied fluids throughout the entire experiment.

Results are summarized in Table 4. Significant plasma concentration as evidenced by plasma chloride determinations occurred, though of course this could not be carried to the same extremes as *in vitro* experiments. In blood smears made before dehydration took place only 1 target corpuscle was seen in many random fields. In blood smears made at the time of maximum dehydration an average of 2 to 4 target cells were seen in every high power field with as many as 6 seen in some and as few as 1 in others. Figure 2 shows photomicrographs of blood smears made before and after dehydration.

TABLE 4.—EFFECT OF RAPID DEHYDRATION ON THE MORPHOLOGY AND VOLUME OF ERYTHROCYTES OF THE DOG

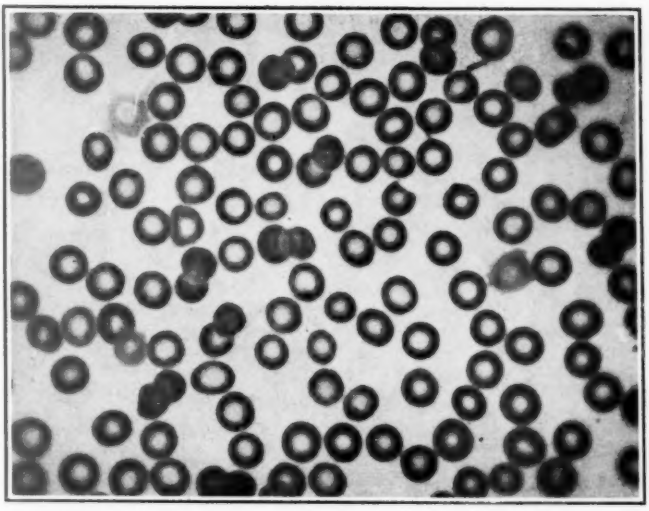
	Elapsed time in hours from start of experiment		
	0 hrs.	8 hrs.	30.5 hrs.
Weight in kg.	13.60	12.48	11.31
% net weight loss	0	8.30	17.00
RBC in millions	5.73	6.34	6.99
Hb. in gm. per 100 cc.	13.40	14.40	15.60
Vol. packed RBC	42.50	42.80	45.00
M.C.V. in c.μ	74.20	67.50	64.40
M.C.H. in $\gamma\gamma$	23.40	22.70	22.30
M.C.H.C. in %	31.50	33.60	34.70
Plasma chlorides, millieq.	116	123	124
Smear, target cells	Very rare	Av. 1 per h.p.f.	Av. 2–4 per h.p.f.

EXPERIMENT 5. In this experiment, an attempt was made to convert naturally occurring target cells in the blood of a patient with sickle cell anemia to cells of relatively normal appearance by suspending them in the patient's own plasma rendered hypotonic by the addition of distilled water.

Twenty cc. of venous blood was drawn from M. W. (No. 195904), a patient with sickle cell anemia, whose blood had been found repeatedly to contain large numbers of target cells. Exactly 5 cc. was placed in each of 4 control hematocrit bottles, and control smears were made from one such sample. Three samples were then centrifuged, and as much of the supernatant plasma as possible pipetted off. A plasma chloride determination was done on the first of these to serve as a

* We are grateful to Dr. E. Adolph for the opportunity of studying this animal during dehydration.

control. The remaining two samples of plasma thus obtained were diluted with distilled water and then added to their respective sedi-



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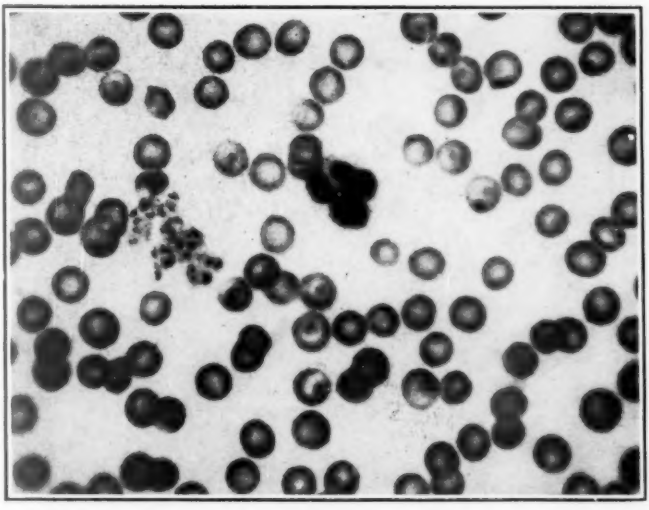

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FIG. 2.—*A*, Blood smear before dehydration of experimental dog (Table 4). (× 970.) Plasma chlorides, 116 milliequivalents.

B, Blood smear of the same animal after dehydration. The field shown represents an area in which the change was maximal. (× 970.) Plasma chlorides, 124 milliequivalents.

ments of red cells in volume equal to the amount of normal plasma previously removed. The cells were resuspended, and smears drawn from this hypotonic blood. These cell suspensions were then recentri-

fuged, the supernatant hypotonic plasma removed, and plasma chloride determinations made on each sample to evaluate the degree of

A

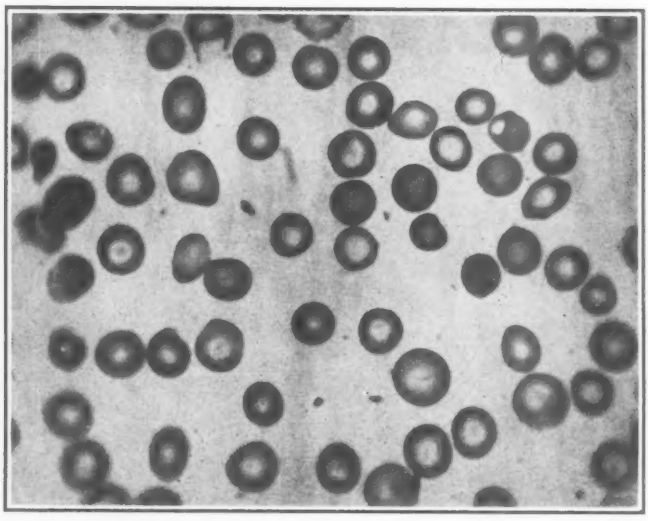

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FIG. 3.—*A*, Blood smear of M. W. (No. 195904), a patient with sickle cell anemia. Note the large number of target cells. (× 970.) Plasma chloride, 106 milliequivalents. *B*, Blood smear of M. W. after suspension of centrifuged erythrocytes in the patient's own plasma rendered hypotonic by dilution with distilled water. Note the almost complete lack of target cells and presence of spherocytes. (× 970.) Plasma chlorides, 70 milliequivalents.

dilution. All smears made from hypotonic blood showed a marked reduction in the number of target cells, a marked increase in the num-

ber of cells of relatively normal appearance, and the presence of spherocytes which were not noted in the control smear. The results are shown in Table 4. Photomicrographs of smears made on control and hypotonic blood are shown in Figure 3.

TABLE 5.—EFFECT ON THE MORPHOLOGY OF NATURALLY OCCURRING TARGET CELLS IN SICKLE CELL ANEMIA OF SUSPENDING THEM IN THE DILUTED PLASMA OF THE SAME PERSON

Smear	Presence of target cells	Plasma chlorides (millieq.)
Undiluted control sample	Very many	106
Diluted Sample No. 1	Fewer target cells; some spherocytes	75
Diluted Sample No. 2	Only occasional target cells; more spherocytes	70

Analysis and Interpretation of Data. Experiments 1, 2 and 3 indicate that artificial target cells indistinguishable in the stained smear from those found in certain pathologic conditions can readily be produced by merely suspending normal erythrocytes in hypertonic plasma or serum. It is immaterial whether the hypertonicity is attained by adding potassium oxalate or sodium chloride to samples of blood, or whether cells are suspended in serum made hypertonic by evaporation without addition of chemicals. Experiment 1 shows that such artificially produced target cells have no increased resistance to hypotonic solutions of sodium chloride, unlike most of those occurring naturally in pathologic states.

Experiment 4 suggests that target cells may be produced *in vivo* in the dog if the plasma can be sufficiently concentrated as a result of rapid dehydration. Here, the plasma of the experimental animal has been rendered hypertonic in comparison with the plasma of the same animal before dehydration. The fact that target cells were not present in such dramatic numbers as *in vitro* experiments is probably due to the limitations in the amount of plasma concentration which can be attained without causing the death of the animal. It seems likely that the degree of cell shrinkage produced *in vivo* in this instance represented approximately the borderline at which the erythrocytes in question first began to assume the form of a target in the blood smear. Further confirming experiments are indicated.

Experiment 5 indicates that target cells occurring in large numbers in a pathologic state, sickle cell anemia, will not appear as targets when suspended in hypotonic plasma.

It is well known that the suspension of erythrocytes in hypertonic solutions results in the loss of fluid from the red blood cell with ultimate crenation, whereas the suspension of erythrocytes in hypotonic solutions results in accumulation of fluid within the cell by a similar process with ultimate lysis. It appears that the artificial target cells produced in Experiments 1, 2, 3 and 4 resulted from cell shrinkage of appropriate degree, and that the disappearance of naturally occurring target cells in Experiment 5 was the result of increased content of intracellular fluid. The former occurred at some degree of hypertonicity before crenation was apparent; the latter at some degree of hypo-

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tonicity before lysis took place. This is further evidence in support of the thesis originally stated by Barrett,[1] that cells whose volume is abnormally small in relation to the cell envelope may appear as targets in the stained smear, and suggests that only an increase in this volume is necessary to make them no longer appear so. Target cells whose envelopes are poorly filled in an isotonic medium, namely, the circulating blood, will imbibe more fluid before hemolysing than will the normal erythrocyte. Consequently they can be expected to be more resistant than the latter to hypotonic solutions of sodium chloride. On the other hand, artificial target cells are poorly filled only while in hypertonic solutions, and will resume their original volume-envelope ratio when again placed in an isotonic environment. For this reason, they show no increased resistance to hypotonic solutions of sodium chloride.

The presence of target cells indistinguishable from one another in solutions of relatively varied hypertonicity (Table 3) suggests that cells whose volume-envelope ratio varies in considerable degree can show the same appearance in a stained smear. It seems logical to infer that cells seen as targets in smears made directly from peripheral blood may in a similar manner have considerable variation in their volume-envelope ratios and still appear as targets. Such variations would be accompanied by varying resistance of these erythrocytes to hypotonic solutions of sodium chloride.

The Significance of the Target Cell. Target cells after all derive their name solely from their two-dimensional resemblance to a "bulls eye" in the stained film of blood. There is as yet no evidence which enables one to differentiate the target corpuscle of sickle cell anemia from that of thalassemia or from that seen in young and otherwise healthy adults with infectious hepatitis. The latter frequently show no alteration in their erythrocyte counts or hemoglobin values. It is just as logical on the basis of our present knowledge to ascribe fundamental importance to the target cell in one as in the others.

We cannot agree with Bohrod[2] that the target cell is only a young, resistant cell appearing in the blood stream as a response to blood loss. We have recently had the opportunity of observing the blood smears of 2 persons with severe, acquired hemolytic anemias and normal erythrocyte fragility tests. In 1 instance a concomitant reticulocyte count was 82% and in the other 62%. Here there was ample evidence that the bone marrow was pouring large numbers of young cells into the peripheral circulation, yet virtually no target cells were seen. We have seen many similar but less dramatic instances in which there was evidence of blood loss and very active erythropoiesis without increased numbers of target cells in the blood smear.

Dameshek[3] has suggested that the target corpuscle might be the fundamental inherited defect in thalassemia. However, since a cell can assume the form of a target merely by being incompletely filled, it seems illogical to regard such a cell in a smear as fundamentally defective. These corpuscles may be the result of defective formation in the bone marrow or of inadequate supplies of erythrocyte building material. but on the other hand they may enter the blood stream as perfectly normal cells and have their intracellular contents altered by factors

present in the peripheral circulation. It is recognized that the reticulocyte may represent a regenerative response to intravascular hemolysis, hemorrhage, or the administration of iron or liver factors to appropriately deficient persons, and is not pathognomonic of any of these. Likewise there are many conceivable mechanisms by which erythrocytes may be poorly filled and appear as target cells in the blood smear. Nevertheless, while we cannot subscribe to such specificity for a corpuscle so widely distributed in a variety of conditions, its prominence in both thalassemia major and minor furnishes food for speculation.

Target cells in large numbers have been often noted in a hypochromic group of anemias and in steatorrhea. The former constitutes in general the group of anemias which respond to iron therapy; the anemia seen in the latter is frequently due to iron deficiency. Thalassemia major and minor belong fundamentally to the group of hypochromic anemias, but differ from other hypochromic anemias in that they remain unaffected regardless of the amount of iron administered. Indeed, according to Wintrobe,[13] they constitute the only hypochromic anemias in which this is true. The prevalence of target cells throughout this group, the close analogy between the blood picture in certain iron deficiency anemias and thalassemia minor, and the unexplained occurrence of large amounts of iron containing pigment in the parenchymal cells of certain viscera in fatal cases of thalassemia major combine to suggest the possibility of some common denominator for the entire group.

It may be that all are, in truth, iron deficiency anemias, and that in some instances the lack of adequate building materials for hemoglobin results in the production of cells whose envelopes are large in proportion of their contents, and which appear as target cells in the blood smear. In the case of thalassemia major and minor, however, lack of available iron may not be due to a depletion of body iron reserves or to an inadequate exogenous iron supply, but rather to the inability of the body to metabolize completely more than a limited amount of iron to hemoglobin regardless of the amount available. The fundamental defect in such a case would be an inherited deficiency of some factor necessary for complete synthesis of hemoglobin from its precursors, the deficiency being quantitatively far more severe in the fatal form of the disease than in its companion, milder, genetically related anemia. It is conceivable that iron converted to the stage of some pigment precursor of hemoglobin, and incapable of being metabolized further, is deposited in certain viscera much as incompletely metabolized products of tyrosine are deposited in cartilage in ochronosis.

The other changes which comprise the striking picture of the child with thalassemia major may be of a secondary and non-specific nature. Erythroblastosis may be the result of the extreme degree of iron deficiency present; the jaundice, which is never marked, may be due to the tendency of abnormally shaped erythrocytes to be more rapidly destroyed than normal erythrocytes, regardless of the cause of the abnormal shape. The changes in the bones are similar to those seen in sickle cell anemia and some cases of familial hemolytic jaundice. These have in common a markedly hyperplastic bone marrow existing continuously from an early date.

This is admittedly speculation but has some theoretical justification. It offers a somewhat more optimistic investigative approach in that the deficient factor may conceivably be found and supplied exogenously. In spite of the long and fruitless search for such a factor, particularly by Whipple and Bradford,[11] it is felt there is reason to continue investigation along this line.

Summary. 1. Target cells may be produced *in vitro* by suspending normal erythrocytes in plasma or serum rendered hypertonic either by the addition of chemicals or by evaporation. Severe, rapid dehydration of a dog, with attendant plasma concentration, resulted in a slight but significant increase in the number of target cells in the peripheral circulation.

2. Naturally occurring target cells in a pathologic condition, sickle cell anemia, may be converted to cells of relatively normal appearance by suspending them in the patient's own plasma rendered hypotonic by dilution with distilled water.

3. The target cell is considered to be a cell whose envelope is large in relation to its contents, regardless of the cause of this relationship. Its wide distribution in a hypochromic group of anemias which respond to iron and in the hypochromic anemias of thalassemia major and minor which do not respond to iron suggests the possibility that the latter may be due to iron deficiency. This iron deficiency might be due to some inherited inability to metabolize completely to hemoglobin more than a limited amount of iron.

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A CASE OF ECZEMA AS A SOURCE OF A STREPTOCOCCAL EPIDEMIC*

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This is a report of an epidemic of streptococcal infection which occurred among nurses working on the Pediatric floor of the Isolation

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Pavilion of the New Haven Hospital during the summer of 1942. Although bacteriologic studies done at the time were incomplete, the clinical and bacteriologic findings pointed to a child with infected eczema as a likely source of the epidemic. The importance which such cases of infected eczema hold in the general pattern of streptococcal infections in childhood has been the subject of a recent report by Boisvert and Powers,[2] and the main purpose of the present paper is to record the circumstances under which a patient with eczema can apparently be instrumental in infecting so many contacts over such a long period of time.

Case Report. F. E., a boy of 2, was admitted with a severe streptococcal skin infection. Three days after a normal birth he had developed a vesicular skin disease which eventually became pustular and was treated with ammoniated mercury. After several weeks the skin cleared up. At the age of 8 months, he developed a dry red rash of the popliteal and anticubital fossæ. The rash later became vesicular and his temperature rose to 105°. He was given one of the sulfonamides, but this treatment was stopped because a generalized rash appeared which was thought to be due to drug sensitivity. Following this illness, until his admission to the New Haven Hospital 1 year later, the skin had never been clear. Various dietary régimes and local treatments had been tried, but the skin remained moist and cracked and peeled extensively.

In January 1942, the patient was sent to St. Mary's Hospital in Waterbury, Conn., with generalized eczema from head to toe, and an upper respiratory infection. A month later, in February, he was discharged improved, but shortly after returning home the eczema became more active and pustules appeared on his legs and buttocks. This seemed to be helped by tincture of merthiolate, but he became very restless and irritable, and on April 11, 1942, was sent to the New Haven Hospital and admitted to the Isolation Pavilion of the Pediatric Service.

On admission his temperature was normal. His body was covered with a fiery-red eczematous rash. The skin was peeling and there was generalized adenopathy. In the scalp there was a moist crusting lesion and over the face, trunk and extremities, a puffiness of the skin. There were scattered papules over the lower extremities and buttocks. The left external auditory canal was filled with a foul discharge. The drum was not seen. The Kahn test was negative. The urine showed occasional albuminuria and transient W.B.C. The R.B.C. and hemoglobin were normal. The total white count on admission was 48,700 with 53% polys. Total serum proteins were 3.4% but later rose to 6.2%. Serum cholesterol was 165 mg. per 100 cc. and the blood chlorides 109.6 mg. Throat and nose cultures were negative but a Type 14 hemolytic streptococcus was recovered from the skin. Stool examination and a chest Roentgen ray were negative.

The child remained desperately ill for 1 month. Then the course of his illness became one of intermittent remissions and relapses, but with steady improvement. Bouts of fever recurred, generally associated with the appearance of widespread, oozing, pustular lesions from which hemolytic streptococci could be cultured. Whole blood and plasma transfusions were given frequently. Evaporated goat's milk and cane sugar seemed to be the best diet. Carrots, liver, rice, pablum, lamb, peas, beans and other foods were added slowly, and shortly before discharge he was changed to whole boiled cow's milk.

Of the various therapeutic measures tried, ultraviolet light, sulfathiazole ointment and sulfadiazine by mouth seemed to be the most effective.

He was discharged October 5, 1942, much improved, although his skin was still rough and scaly and slightly erythematous in the intertriginous areas. Cultures of his skin were negative at this time.

On October 29, 1942, he was readmitted. He had gone steadily downhill since discharge. On readmission he had Type 14 hemolytic streptococci in

his nose and throat cultures as well as in cultures taken from his skin. Penicillin soaks were beneficial but had no effect on other bacteria, notably one of the Friedländer group of organisms cultured from his skin. He was put on a Rowe diet, coal-tar ointment and sulfathiazole ointment. As it was the consensus of those who directed his care that his tonsils might be a factor in reinfecting his skin, a tonsillectomy and adenoidectomy were done Jan. 20, 1943. He again improved and on February 6 was discharged. His skin was clearer than it had been since he was 8 months old. There was no oozing, inflammation or pustules. Cultures of his skin were negative at this time.

Description of Streptococcus Epidemic. The pediatric floor of the Isolation Pavilion has 2 twelve bed wards on either end of the floor, with 4 single rooms, 2 double rooms and nurses' offices between. Isolation precautions are observed, a clean gown being worn by all those attending each patient. Hands are washed in soap and water and rinsed in lysol solution after visiting a patient. Dishes are steam sterilized.

On June 2, 1942, a house officer who was one of 4 working on the floor became ill with a streptococcal reinfection of an old osteomyelitis sinus tract. On June 3, 2 student nurses came down with scarlet fever. Suspicions were aroused and work was begun in an effort to determine the origin of these infections. There were 20 patients on the floor at the time (4 of whom were or had been ill presumably with streptococcal infections), and as attendants, 27 student nurses, and 2 graduate nurses. All the adults were in good health.

Cultures were taken on June 6 on the 4 pediatric patients with streptococcal disease, but only 1 (F. E.), the child with eczema described above, had a positive culture, the other 3 being convalescent. From F. E.'s scalp, a Group A, Type 14 hemolytic streptococcus was isolated—an organism which had previously been isolated, at the time of his admission. During the next 2 months we cultured the throats of 13 nurses who left the floor with sore throats or scarlet fever, 7 of whom proved to have Group A, Type 14 hemolytic streptococci on immediate typing. The remaining 6 we were unable to type.

It seemed significant to us that F. E. was the only acutely ill child on the floor with a streptococcal infection, and the only one from whom we were able to culture hemolytic streptococci. Because of his restlessness and the severity of his infection he needed constant special nursing care. All student nurses had their turn taking care of him. The bed and the floor beneath the bed were covered with desquamated skin.

The number of student nurses on the floor at any one time varied between 15 and 30. A total of 74 worked on the ward during May, June and July. A few were from the Yale School of Nursing, but most of them were affiliate nurses from other hospitals, arriving in groups of 3 to 12, and remaining from 4 to 6 weeks, depending upon the hospital from which they came.

During the months of June and July, 11 other juvenile patients with streptococcal infections were admitted to the ward. Four were Type 28, 2 Type 26, 2 Type 12 and 1 each of Types 11, 25 and 27. None was on the ward for more than a month except 1 case of osteo-

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myelitis, with streptococcal otitis media. He was discharged in 6 weeks.

Subsequent to June 3, 1942, no more cases of scarlet fever developed among the nurses for a few days, but several went off duty with what was called an "upper respiratory infection." Unless epidemiologic studies were being done, probably many of these cases of sore throat among the nurses would not have been studied bacteriologically. The illness was mild, rarely with a temperature over 100°, and seldom lasting more than 4 or 5 days. The throat was sore but not striking in appearance, nor was there any extreme degree of malaise and anorexia.

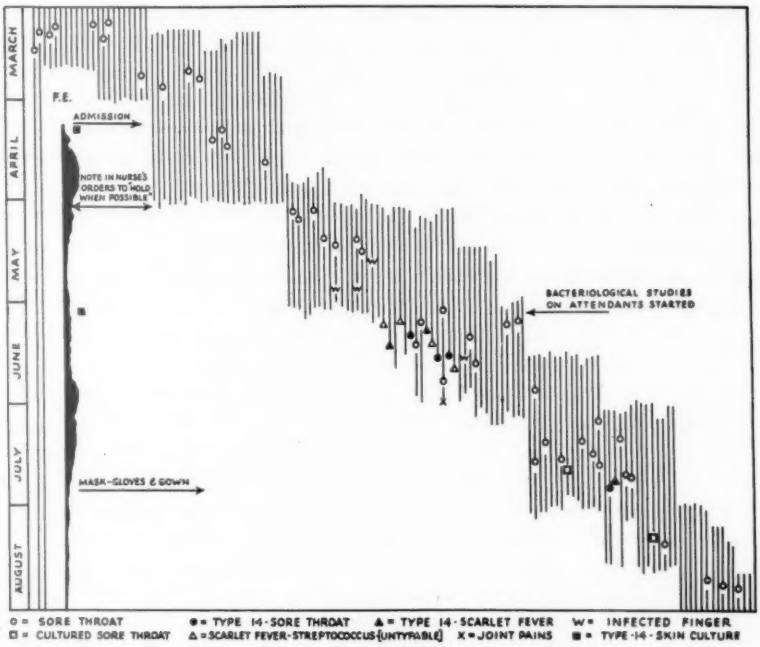

CHART 1.—Relation of F. E.'s fever to nurses' illness. Recorded vertically on the left border of the chart in solid black is the course of F. E.'s temperature and pertinent nurses' orders. Each parallel vertical line (occupying the oblique zone across the chart) represents the duration of each nurse's duty on the ward. The legends on these lines represent the time when they became ill and the character of the illness—see explanation at the bottom of the chart.

As we were able to culture Type 14 streptococci from some of these cases, it seemed that they were part of the same ward epidemic and consequently sickness records of the student nurses before F. E.'s admission were examined to compare the incidence of possible or proven cases of streptococcal disease before his admission with the incidence after his admission.

These data appear in Chart 1. In March and April, before F. E.'s admission, the prevalence of "upper respiratory infections" among the student nurses was 22%. Of 44 nurses, who had been on the floor, 10 had gone off duty with upper respiratory infections. None left for

other illnesses. All of these illnesses had been mild and brief, and no cultures had been taken. During the *epidemic* months of May, June and July, 74 nurses worked on the floor, 37 (50%) of whom became ill with an upper respiratory infection, scarlet fever, or a skin infection. We were not able to take cultures on all these, as some of them were sick in May, before our studies began, and others who became ill after our studies began were affiliate nurses and returned to their own hospital. We were able to culture 13 of the 37, 7 of whom had scarlet fever, and 6 of whom had sore throats.

It is difficult to find a close relationship between the course of F. E.'s illness and the epidemic but as long as he ran some fever the attendants taking care of him became ill. When his infection became less active and after the nurses had begun to wear masks and gloves as well as gowns the epidemic subsided.

We questioned F. E.'s father concerning the health of the family prior to the child's admission to the hospital and learned that 2 aunts and a grandmother had had skin infections of the neck and arms and sore throats after they had begun to help take care of him. His mother who visited him frequently at the hospital came down with scarlet fever in June 1942. We were unable to get throat cultures as she lived in a town some distance away. The patient's father had a streptococcal infection of the nares 6 weeks before F. E.'s admission.

TABLE 1.—TYPING RESULTS IN 14 CASES

		Typing		
		Yale*	RH*	Led.*
	F. E.	14	..	Untypable
Nurses and interne	Wlc.	14	Untypable	Untypable
	Lgn.	14	Anti-T 14	Untypable
	Jd.	14	..	Untypable
	He.	14	Anti-T 14	
	Sdr.	14		
	Hz.	14		
	Qn.	14		
	Ps.	Untypable	..	Untypable
	Bn.	Untypable	..	
	Js.	Untypable	..	Untypable
	Mr.	Untypable	..	Untypable
	Bly.	Untypable	..	Untypable
	Cld.	Untypable	..	

* Yale = typing done (early in the epidemic) at Yale University School of Medicine. RH = typing done at Rockefeller Hospital. Led. = typing done at Lederle Laboratories.

Bacteriologic Studies. In all, 14 cases of possible streptococcal infection were cultured, from 13 of which Group A organisms were isolated. The other was a Group G organism. Throat swabs were inoculated in beef broth and later streaked on rabbit blood agar plates. Two types of colonies grew out on the plates—one, a typical matt, hemolytic colony, and the other a minute anhemolytic, slightly green colony. Of the 2, the small anhemolytic colony was more common. However, on further subculturing, these would often revert to the typical matt forms. In rabbit blood broth, these organisms produced a greenish hemolysis and fairly granular growth. They were bile-insoluble, and microscopically were typical chain forms.

In Table 1 we have collected data on typing. These data would indicate to us that many of the cultures were somewhat unstable as far as the retention of their type-specific properties was concerned. Of the 13 Group A strains, 7 proved on immediate slide agglutination to be Type 14 and 6 were untypable. Two sets of typing sera were used, one furnished by the Department of Pediatrics, and the other by the Lederle Company. Subsequently, however, when 3 of the "Type 14" strains were checked by Mrs. Rebecca Lancefield of the Rockefeller Institute for Medical Research and by the Lederle Laboratories, they were found untypable. Mrs. Lancefield used the precipitin reaction and the Lederle Laboratories the slide agglutination technique. Because some epidemic strains have been found to contain Type 14 T, but no Type 14 M, Mrs. Lancefield tried to type the 3 strains we sent her with a serum containing Type 14 T antibodies. Two of the strains gave positive agglutinations with this serum.

None of the strains was studied for erythrogenic toxin production.

The results of rabbit immunization done by us as well as by Mrs. Lancefield and the Lederle Laboratories gave inconclusive and irregular results.

Discussion. Coburn[3] has called attention to the fact that hemolytic streptococcal infection in infants can acquire a high degree of communicability, and in a recent paper by Boisvert and Powers,[2] attention is called to the possible endogenous source of skin infection in children with eczema and concomitant streptococcal fever. Events described in this paper appear to bear out these observations, and illustrate another point, that secondarily infected eczema in a child can be the source of a veritable epidemic of streptococcosis. As was the case in an epidemic studied by Coburn, we found no evidence of sick nurses transmitting the disease to others, and particularly roommates, during the incubation period. In this respect this epidemic was also centered about its source behaving somewhat as a milk-borne epidemic of streptococcus disease in which groups of new susceptibles are continually being exposed to a heavy "common source" of infection but who fail to pass along the disease to their contacts.

This epidemic also furnishes an illustration of the variegated forms which streptococcus illness may assume, in which about 50% were cases of scarlet fever, 50% sore throats. This, in other words, is an example of the concept of Boisvert and Powers of streptococcosis.[1]

It is noteworthy that in many of the cultures from different patients the strains gave rise to 2 types of colonies, hemolytic and anhemolytic slightly green colonies. Whether this atypical growth can be related in any way to the difficulty encountered in typing the strains, we are not able to say. While the typing sera used by us has not given false positives as far as we know, it is possible that it contains a high titer of antibody against the particular T substance of this epidemic strain. This has not been determined.

Summary. A small epidemic of streptococcal diseases among student nurses taking care of a child with secondarily infected eczema is reported. The child was a constant source of Type 14 hemolytic

streptococci for many weeks. During a 3-month period, 50% of the nurses who cared for this child became ill with sore throats or scarlet fever.

We believe that either the "dosage" of the organism put out by this child was high, or that the strain was peculiarly infective.

Difficulty was encountered in typing this epidemic strain, which grew both as a typical matt form, and as a small anhemolytic, slightly greenish colony.

We are indebted to Miss Carol Reynolds for assisting us in obtaining data on the nurses. We are also indebted to Dr. John R. Paul for his guidance and assistance in preparing this paper.

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THE EFFECT OF SIMULTANEOUS TUBERCULOUS INFECTION ON EXPERIMENTAL TRICHINELLA INFESTATIONS IN GUINEA PIGS

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Evidence suggesting that an unrelated disease process may alter the interpretation of the trichinella skin test was uncovered in the course of investigation on the incidence of trichinosis in North Carolina. In an attempt to determine whether the low incidence of trichinosis in diaphragms obtained at autopsy in this state (2.8%) might be due to light infestations which were undetected after death, a study was made to determine the incidence of positive reactors to skin test antigen.[4] In the course of this study, it was noted that patients with active tuberculosis in 2 sanatoria gave a higher percentage of positive reactions (14.3%) than did those without tuberculosis (7.1%) in 2 general hospitals.[5] Another study was undertaken to determine whether the high incidence of positive skin tests in patients with tuberculosis could be accounted for by unrecognized subclinical institutional epidemics, or if some biologic factor were involved.[3] In order to keep the institutional factor constant, the control non-tuberculous group was chosen from patients confined in hospitals for equivalent periods of time, and was of necessity composed chiefly of psychiatric patients. Because of the unexpected finding that psychiatric patients themselves gave

abnormal responses to skin tests, the experiment could not answer clearly either of the questions.

In an attempt to discover if a true biologic cross-reaction, detectable by skin test, exists between *Mycobacterium tuberculosis* and *Trichinella spiralis*, or if simultaneous infection with both organisms alters the usual course of either infection, the present experiments were undertaken.

Material. Thirty-three guinea pigs of approximately 250 to 350 gm. each, obtained from a single source at one time, were divided into 4 groups. Each group was confined in a separate cage. All were fed a standard guinea pig diet of food pellets (Rockland guinea pig diet, vitamin C fortified, Arcady Farms Milling Company, Chicago, Ill.); green vegetables were supplied twice weekly. The animals were kept under standard conditions for 2 weeks before being infected.

The strain of *Trichinella spiralis* used was obtained from the National Institute of Health and was carried in rats. The culture of *M. tuberculosis* (human) Strain C was obtained from Lederle Laboratories and was carried on bean-egg media; the tuberculin (Lederle) (K.O.T.) was made from this strain.

Methods. The abdomens of all animals to be tested were shaved. The following skin tests* were given to all animals 4 days before they were infected: (1) purified protein derivative (P.P.D.) of 0.005 mg. in 0.1 cc.; (2) 0.1 cc. of 1:100 dilution (1 mg.) of tuberculin (K.O.T.) (Lederle); (3) 0.1 cc. of 1:100 dilution (1 mg.) of tuberculin (K.O.T.) (Saranac); (4) 0.05 cc. of 1:10,000 trichinella extract; (5) 0.05 cc. of 1:8000 trichinella extract; (6) 0.05 cc. of buffered saline diluting fluid as a control. The skin tests were read at 20 minutes, 24 and 48 hours. New syringes and needles were obtained for the skin tests and were not used for any other purpose. Each test solution was always given with the same syringe and needle; the syringes and needles were never interchanged. After being used, they were washed with distilled water, placed in marked glass tubes and sterilized in an autoclave.

Group A, consisting of 7 animals, were fed trichinella larvæ, Group B (8 animals) were injected with tubercle bacilli, Group C (9 animals) were fed trichinella larvæ and were injected with tubercle bacilli, and Group D (9 animals) served as controls.

Eight weeks after infection—4 weeks before the animals were sacrificed—the skin tests were repeated, using different sites in the skin of the abdomen.

A rat infected with trichinæ for 8 weeks was killed by a blow on the head; the muscle was dissected away with scissors, ground, and digested at 37° C. for 4 hours in a solution of 0.7% of pepsin and 1% commercial hydrochloric acid. The digested mixture contained 1 to 2 live larvæ per drop. Each animal in Groups A and C were fed 1.5 cc. of this suspension by glass dropper. The mixture was thoroughly agitated between each withdrawal.

One loopful of the culture of tubercle bacilli was emulsified in 5 cc. of sterile saline. On smear this suspension showed 10 to 20 organisms per oil-immersion field; 0.05 cc. was injected into the groin of each animal in Groups B and C on the day following the feeding with trichinæ.

Those animals which had not died were sacrificed 12 weeks after infection and autopsies were performed. Smears were made from the tuberculous lesions in liver, lungs, lymph nodes and spleen and were stained with carbol-fuchsin by the Ziehl-Neelsen technique. The results were recorded according to the Gaffky scale. The entire diaphragm was compressed in a muscle press made of 2 pieces of heavy plate glass and the whole diaphragm examined under a microscope using a 10× objective and 12.5× ocular. If this examination

* The purified protein derivative (P.P.D.) was furnished by Sharp & Dohme; tuberculin (K.O.T.), trichinella antigen 1:10,000 and diluting fluid by Lederle Laboratories; trichinella antigen 1:8000 by the National Institute of Health. Tuberculin (K.O.T.) was also obtained from Saranac Laboratory.

was negative, approximately 1 to 2 gm. of skeletal muscle from various parts of the body were minced with scissors and similarly examined. The infections were recorded as minimal (1+) if the diaphragm was negative, but larvæ were found in other muscle; mild (2+) if less than 1 larva occurred per field; moderate (3+) if the average was 1 per field; heavy (4+) if 2 or more were seen per field.*

TABLE 1.—DENSITY OF ORGANISMS IN TISSUE

	Group A Trichinosis		Group B Tuberculosis		Group C Trichinosis and tuberculosis		Group D Control	
Animals	Trichinæ*	Tubercle† bacilli	Trichinæ	Tubercle bacilli	Trichinæ	Tubercle bacilli	Trichinæ	Tubercle bacilli
1	+‡	0	0	+	++++¶	+	0	0
2	+	0	0	+	++++	++	0	0
3	+	0	0	+	++++	+	0	0
4	++§	0	0	+	++++	+	0	0
5	++	0	0	++	++++	+	0	0
6	++	0	0	++	+++	++	0	0
7	++	0	0	++	++	+	0	0
8	..	..	0	++	++	+	0	0
9	..	..	..	..	++	++	0	0

* + indicates 0 trichinæ in the diaphragm, but larvæ in other muscle, <1 gm. ++ indicates <1 larva per microscopic field (× 125) in muscle press of diaphragm; approximately 1–533/gm. +++ indicates 1 larva per microscopic field (× 125) in muscle press of diaphragm; approximately 400–800/gm. ++++ indicates 2 or more larvæ per microscopic field (× 125) in muscle press of diaphragm; approximately >800/gm.
‡ Diaphragm and skeletal muscle negative in muscle press, digestion positive.
† Gaffky scale, carbolfuchsin stained smears.
§ Died of streptococcal abscesses.
¶ Died of trichinosis.

Results. The preliminary skin tests were all negative. All of the animals injected with tubercle bacilli gave positive tuberculin skin reactions at 48 hours when tested 8 weeks after inoculation; no others reacted. In spite of reports in the literature[1] that guinea pigs will react to trichinella skin test, only 1 animal which was fed trichinæ gave a questionable positive skin reaction to the National Institute of Health antigen; all other reactions to trichinella antigen were negative. One antigen (Lederle) had been used extensively in human beings and found satisfactory. At autopsy, acid-fast tubercle bacilli were demonstrated in smears from the tissues of all animals inoculated with tubercle bacilli. Trichinæ were demonstrated in the diaphragms or skeletal muscles of all animals fed trichinæ. In the control groups the skin tests and examination of tissues were negative.

One animal in Group A died 10 weeks after infection of streptococcus septicemia with generalized abscess formation, but the trichinella infestation was no heavier in this animal than in the remainder of the group. All of the animals in Group B, injected with tubercle bacilli alone, survived for the duration of the experiment. One animal in Group C, infected with both trichinæ and tubercle bacilli, died 10 weeks after infection, apparently with overwhelming trichinosis. The remainder lived for the duration of the experiment.

* Since there are at this magnification approximately 800 fields in a diaphragm of 1.5 to 2 gm., the number of organisms per gram would be roughly: less than 1 in the mineral infestations (interpreted as 1+), 1–533 in the mild infestations (2+), 400–800 in the moderate infestations (3+), 800 or more, in the heavy infestations (4+).

Each of the 7 animals in Group A, infected with trichinæ alone, had a light (1+) infection at autopsy. In 4 the diaphragm was positive (2+); in 3 the diaphragm was negative, but trichinæ were found in skeletal muscle (1+). In 1 of the latter group (1+) the muscle press preparations were negative, but trichinæ were recovered by digestion. Smears taken from infected areas of the tuberculous animals in Group B were equally divided between those showing Gaffky 1 and those showing Gaffky 2 density of organisms. In Group C the smears of tuberculous lesions showed 6 Gaffky 1 and 3 Gaffky 2 density of organisms; this was comparable to Group B, infected with tubercle bacilli alone.

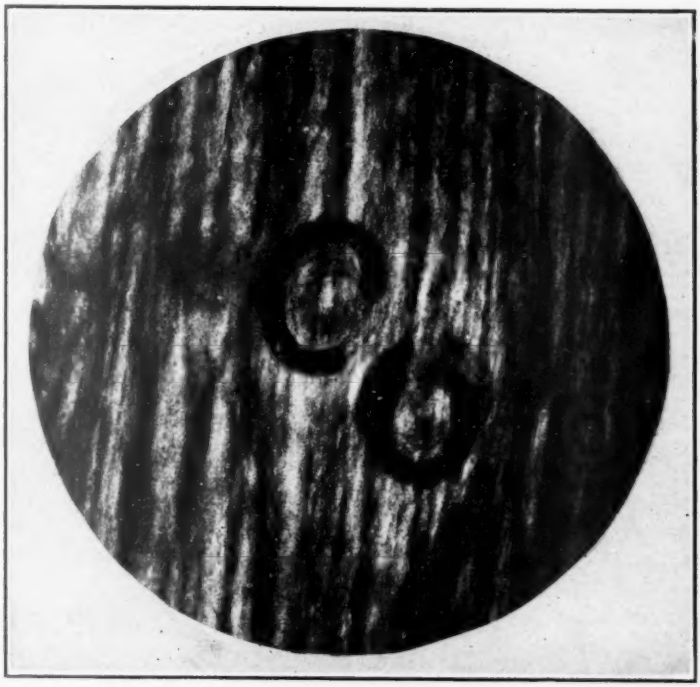

FIG. 1.—Typical field in a muscle press preparation of diaphragm in experimental trichinosis. This density of infection, seen with 10× objective and 12.5× wide-angle ocular, is the minimum for ++++ density in the table.

The density of trichinella infestation, however, was strikingly increased in most of the animals in Group C; parasites were found easily in each of the diaphragms. Three animals showed mild (2+) infestations comparable to those in Group A; 1 animal showed a moderate (3+) infestation; 5 animals showed a heavy (4+) infestation with trichinæ; this density was not found in any other group.

Treating the data as scored (1, 2, 3, 4+), the mean difference between Groups A and C gives a relative deviate of $t = 4.02$, with $P < 0.01$. If 1+ and 2+ are grouped as light, 3+ and 4+ as heavy, by the chi square method $X^2 = 5.666$ and $P > 0.01 < 0.05$. By an exact

FIG. 2.—Trichinæ recovered from digestion of muscle in experimental trichinosis. This density of organisms is comparable to the minimum for ++ density in the table, and is comparable to Group A.

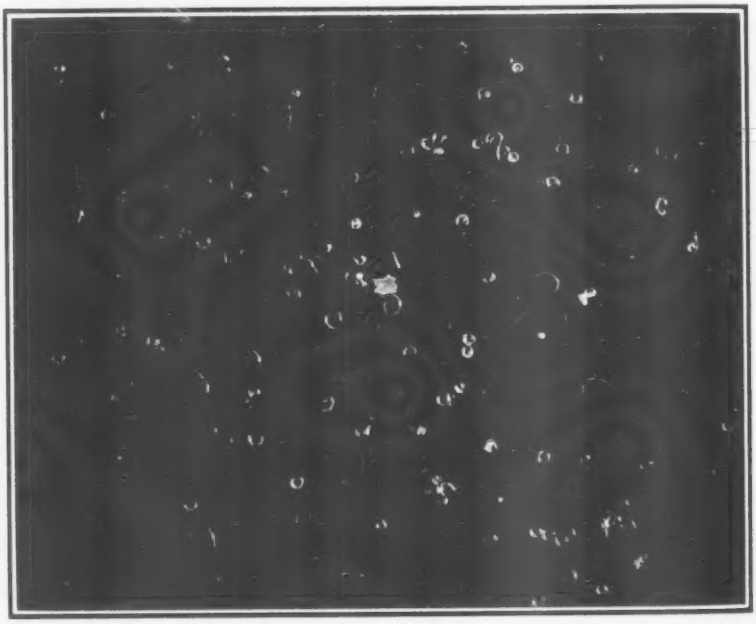

FIG. 3.—Trichinæ recovered from digestion of muscle in experimental trichinosis. This density of organisms is comparable to the minimum for ++++ density in the table, and is comparable to Group C.

probability method the same contingency (2 x 2) table gives P = 0.03. By either of these methods the data are definitely significant.*

The general health of the pigs in all groups was comparable. Groups A and D gained weight normally and appeared perfectly healthy; Groups B and C gained weight but not to the degree that Groups A and D did. The hair appeared slightly ruffled in each infected group. By clinical observation, it was impossible to differentiate between Group B, infected with tuberculosis alone, and Group C, infected with both trichinosis and tuberculosis.

Discussion. The increase in density of trichinella infestation in animals infected simultaneously with trichinæ and tubercle bacilli has not previously been described. In some instances the infestation in the diaphragm was up to 1600 times as great as in non-tuberculous animals fed the same dose of larvæ.

Spink, studying the eosinophil response in guinea pigs, injected tubercle bacilli into 1 group of animals and 15 days later fed them trichinous meat. He also fed another group trichinous meat and, after the establishment of eosinophilia, inoculated them with the same quantity of tubercle bacilli used in the previous experiment. No attempt was made to quantitate the inoculum of trichinæ. Postmortem examination of stained paraffin sections revealed no differences in the microscopic appearance of either muscle or parasites in the 2 groups of tuberculous trichinous guinea pigs as compared with trichinous guinea pigs. This technique would not allow an estimation of the density of infestation, because of the small amount of tissue on 1 slide.

Spink also studied the effect of killed tubercle bacilli, typhoid vaccine and infections with *Staphylococcus aureus* and *Trypanosoma equiperdum* on the eosinophil count and tissue reaction in trichinous animals.[8] No conclusions can be drawn as to the effect of these organisms on the density of infestation, though differences were noted in the blood picture and tissue response from that in trichinous animals.

It is unlikely that in our experiment either organism assisted directly in the invasion of tissues by the other, since they entered the body by entirely different routes. The trichinæ entered through the intestinal tract and the tubercle bacilli by the lymphatics of the groin. It is possible that the tuberculous infection may have entered the blood stream at the same time that the trichinæ were being disseminated through the blood, and it may have exerted an influence in this fashion. A tuberculous infection in the anergic state may have an indirect effect on the dissemination of particulate matter, as has been shown with dye in the skin of tuberculous guinea pigs.[6] The only animal to die of trichinosis was in the group simultaneously infected with tuberculosis, but the animal reacted to tuberculin.

In sarcoid, a superimposed tuberculous infection has been said to alter the character of the disease.[7] Secondary infection by the tubercle bacillus is known to occur frequently in tissue damaged by silicosis, sarcoid and Hodgkin's disease; the tubercle bacillus may have an affinity for tissue damaged by infestation with trichinæ, but it was not demonstrated by this experiment. If this is true, individuals with

* We are indebted to Sgt. Charles W. Cotterman for statistical criticism of these data.

trichinosis may develop tuberculosis more easily than patients without the parasitic infestation. This would explain the higher incidence of trichinella skin tests in tuberculous patients.

Does tuberculosis predispose the individual to an infection by a smaller number of trichinæ than are required to infect a person without the disease? In the muscle press preparations, trichinæ were not found in areas infected by tubercle bacilli, but were discovered in the usual site, though in far heavier numbers than in the non-tuberculous group of animals. That the trichinella infestation had no effect on the tuberculosis is shown by the fact that the density of tubercle bacilli in Group C was no greater than in Group B, infected with tubercle bacilli alone. Under the conditions of the experiment, it is impossible to say whether tuberculous animals can be infected with smaller doses of trichinæ than can non-tuberculous animals. The dose used in Group A was small, as shown by the light and mild infestations, but may not have been minimal. If tuberculous patients can become infected with smaller doses of trichinæ than non-tuberculous patients, the incidence of positive reactions to trichinella antigen would be greater. Such an explanation would be logical, since most infestations in non-tuberculous patients in this area have been shown at autopsy to be light.[4] Data indicating the minimal degree of trichinella infestation in human beings necessary to produce a positive skin test are not available.

The failure under the conditions of this experiment of the skin of guinea pigs to react to intradermally injected trichinella antigen leaves unanswered the question of possible crossed skin reactivity between *M. tuberculosis* and *T. spiralis*. One antigen (Lederle) had proven satisfactory in our hands in previous human experimentation in the dilution used (1:10,000). The dilution of antigen (1:100) used by Bachman[1] has been thought to give non-specific reactions which can be avoided by a greater dilution;[2] hence, the more dilute solutions were used in this experiment.

Summary. Guinea pigs infected simultaneously with *T. spiralis* and *M. tuberculosis*, when sacrificed 12 weeks later, showed heavier infestation with trichinæ than did similar animals infected with trichinæ alone.

The converse of this statement was not true, since the density of acid-fast organisms found on smears from lesions was no greater than that observed in similar animals infected with *M. tuberculosis* alone.

An increased density of infestation with trichinæ in the presence of active tuberculosis may account for the previously reported finding of a higher incidence of positive reactions to trichinella antigen in tuberculous patients confined to sanatoria than in comparable groups confined in general hospitals.

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CLINICAL ASPECTS OF PAIN IN THE CHEST
II. Pain Arising From the Esophagus

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During the past 2 decades both the medical profession and the lay public have become increasingly aware of the frequency and seriousness of diseases of the coronary arteries. The erroneous assumption is often made by the patient, and occasionally by the physician, that any pain which originates in the substernal or precordial areas—and more especially if it radiates to the left upper extremity—is necessarily due to disease of these vessels. The physician who is particularly interested in cardiac disorders is likely to be concerned only with the question as to whether the pain of a given patient is due to abnormality of the heart, and once he is satisfied that this is not the case often loses interest in the actual cause of the pain. It is not enough to know what does not produce the pain, but one should also know what does produce it. The purpose of this series of studies is to investigate points of differential significance between the various diseases which cause pain in these areas. Especial emphasis is placed on a study of the qualities of the pain, because in some instances the patient's story of the pain offers the only means of diagnosis, and in practically all subjects the history furnishes the essential clue to those further studies which may be needed in order to establish the diagnosis.

The present and succeeding publications deal with disorders of the alimentary tract as causes of chest pain. The frequency of these disorders in causing discomfort in this area of the body is illustrated by the fact that in a series of several hundred patients complaining of this symptom, the cause has been found to be in the alimentary tract in approximately one-sixth of the instances, and in about one-fifth of these the source of the discomfort has been localized in the esophagus. It would appear, therefore, that the esophagus is responsible for 3 to 4% of the instances of chronic or recurrent chest pain.*

The patients considered in this report were questioned in great detail about their discomfort, and in addition were subjected to com-

* The apparent frequency of various disorders as a cause of pain in the chest will naturally vary according to the means whereby the cases are selected. Thus, the patients considered in this study were, in the main, individuals who were seen by the author because they were considered—either by themselves or by their physicians—as suffering from cardiac disease. It is likely that physicians especially interested in diseases of the gastro-intestinal tract, or of the lungs, would observe higher and lower respective frequency of esophageal disorders as causes of the thoracic pain. Likewise, the relative incidence of the various types of esophageal disorders varies according to the special interest of the physician. Thus, only 1 patient in the series had carcinoma of the esophagus. Patients with this disorder usually have symptoms of obstruction prior to the development of pain and, hence, are not suspected of having cardiac disease. On the other hand, patients with those disorders of the esophagus which produce relatively little obstruction, but have substernal pain as the presenting symptom, are likely to be seen by the cardiologist.

plete physical examination and various laboratory studies, including gastro-intestinal Roentgen rays, electrocardiograms and other special procedures as seemed indicated. In patients presenting unusual features, an especial attempt was made to reproduce the pain, because this procedure is often of great value in diagnosis.

Spasm of the Esophagus. Of the 11 patients with whom this report deals, 8 are believed to have had esophagospasm. One of these presented severe pain and an unusual picture, and will be discussed separately. The other 7 patients had milder symptoms and may be included together. Of these 7 patients, 5 were women and 2 were men. Their ages varied from 30 to 66. The pain was mild and was centered in the substernal region in 5 subjects, and in the precordial region in 2 individuals. There was relatively little radiation. The discomfort was described as burning by 1 patient, and as either a sense of fullness, tightness or squeezing by the remaining 6. The duration varied from a few minutes to several hours. The discomfort was precipitated by swallowing in 4 of the 7 patients, and each of these complained of a feeling of a lump in the chest after swallowing. The esophagus was negative to Roentgen ray examination in all of the patients, none of them being examined at a time when the discomfort was present. Nitroglycerin had a dubious effect in the 2 patients who received it, but all of the subjects obtained partial or complete relief by the use of atropine.

The resemblance of the pain exhibited by these patients to the pain of angina pectoris is noteworthy. This resemblance included the location, the duration and the quality of the pain. However, the precipitating factors were entirely different in that in none of these patients was the pain brought on by exertion, although emotion tended to induce it in 5 of the 7 subjects. The mildness of the pain was of little value in excluding angina pectoris, in that a fairly large percentage of the patients with this disorder complained of pain of minimal severity.[4] The similarity of the pain induced by cardiospasm and that caused by disease of the coronary arteries has been commented on by numerous authors and, indeed, some of them[5,9] have even concluded that the pain of true angina pectoris arose from the esophagus rather than from the heart.

The 8th patient with pain due to spasm of the esophagus presented an entirely different picture.

CASE 1. This 47 year old white male, with a history of rheumatic fever, presented the typical findings of stenosis of the aortic valve, and complained of pain arising in the precordium, radiating to the left shoulder and arm, and brought on by physical exertion and by emotion. This pain was relieved by nitroglycerin. In addition he had another and entirely different pain, which was localized to the lower retrosternal region, which lasted from 1 hour to as long as 2 days, and was usually constricting but sometimes stabbing in quality. This pain was quite severe, being somewhat more violent than the anginal attacks. During this second type of pain he often had difficulty in swallowing and a feeling that something was hanging in the region of the lower third of the sternum. At times the difficulty in swallowing, which usually accompanied the pain, was so great that fluids would regurgitate through the nose

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when he attempted to swallow during the attacks. He had noted that these seizures of substernal pain were sometimes precipitated by straining at stool, and were frequently induced by the ingestion of alcoholic beverages. His pain was usually aggravated by deep breathing, but holding the breath did not give complete relief. The termination of his attacks was remarkable. This came suddenly and was associated with a sound which could be heard not only by himself but by others, and which was described as gurgling in character, the sound seeming to arise in the lower sternal region. Immediately following this he felt as though something were "turned loose," and had a warm dripping sensation, internally. Because of the frequency and severity of these attacks of pain the patient had become addicted to morphine.

Physical examination was negative except for the presence of an enlarged heart and of aortic stenosis. Radiologic examination at a time when he was free of pain was negative. When repeated during an attack of pain, marked obstruction was found at the lower end of the esophagus, the appearance being typical of cardiospasm. At a time when he was free from pain a stomach tube was introduced and the stomach was inflated with air. This caused a typical seizure of pain. The following day, after thorough atropinization, the procedure was repeated and three times as much air had to be introduced into the stomach in order to cause the pain as before. The patient obtained marked relief by the use of antispasmodics, but continued to have his pain in milder form when he became emotionally disturbed.

This patient resembles, in many respects, the individual reported by Eideken.[3] His subject, a man of 67, likewise had aortic stenosis. He had pain induced by effort and also pain brought on by swallowing. The former was benefited by nitroglycerin, the latter by belladonna and hyoscyamus. The distribution of the anginal pain and the pain of esophageal origin was apparently identical. Wolferth and Eideken[10] pointed out the frequency with which spasm of the esophagus simulated angina pectoris, and believed that esophagospasm constituted the most frequent condition which led to difficulties in differential diagnosis. They studied a large series of patients with substernal pain, and pointed out that while spasm of the esophagus is often painless, there are some instances in which the discomfort may be very severe. They emphasized the point—well borne out in the case cited above—that it is often necessary to carry out radiologic examinations during the pain in order to detect the esophagospasm.

The chief points which serve to differentiate pain due to esophagospasm from that due to angina pectoris are: (1) the lack of relationship to effort; (2) the frequent relationship to swallowing; (3) the less striking relief from nitrites; (4) the beneficial effect of atropine; (5) the tendency toward longer duration (the pain of angina pectoris does not ordinarily last more than a few minutes[4]); (6) the lack of electrocardiographic changes during the pain; and (7) the demonstration of spasm of the esophagus by radiologic or esophagoscopic examination.

Discomfort Due to the Trapping of an Air Bubble in the Esophagus. CASE 2. A male, aged 52, with a minimal rheumatic mitral lesion and a healed duodenal ulcer, complained of mild substernal discomfort without radiation, lasting for a few seconds. His discomfort consisted of a feeling of fullness and this was quickly followed by a vibratory sensation which he described as feeling "like the nozzle of a garden hose were squirting in the chest." On examination there was a Grade 2 systolic blow at the apex and a faint presystolic murmur when he lay on his left side. At times both heart sounds in the region of the

sternum had a high-pitched, metallic quality. This phenomenon varied with position and at times was not present. Fluoroscopic examination of the esophagus showed that he had a large gas bubble in the stomach and that pressure in the epigastrium forced part of the gas up into the esophagus, where the bubble tended to land. At this time the metallic quality of the heart sounds reappeared, such quality evidently being due to the beating of the heart on the gas bubble. When a stomach tube was passed and the stomach inflated the patient's sensation was not reproduced. However, when the tube was withdrawn into the lower esophagus and air was suddenly run into the tube with a syringe, he perceived a sensation something like the one of which he had complained. When the air in the syringe was mixed with water and the mixture was suddenly forced through the tube into the esophagus the patient stated that the sensation was exactly reproduced.

In this patient, as in so many others, the chief concern was not with the discomfort, which was very mild, but with the patient's interpretation of the significance of the discomfort. Knowing that he had heart disease he was inclined to attribute the curious substernal sensation to a serious cause. When the sensation was reproduced and its mechanism explained, all his anxiety disappeared.

Congestion of the Esophagus as a Cause of Discomfort. CASE 3. A male, aged 50, stated that for 6 months he had had a mild sensation of fullness and tightness in the lower part of the throat. This sensation would come on about 5 seconds after he lay down, would remain while he was in the recumbent posture, and would disappear within a few seconds after he sat up. He had no discomfort on swallowing. Because a relative had recently died from angina pectoris and had had most of his pain in his neck in the midline, the patient was concerned about the possibility of having angina pectoris. Physical examination, electrocardiograms and radiologic study of the esophagus were negative. The story of the curious relationship to posture suggested that increased venous pressure in the esophagus might be concerned with the discomfort. Therefore, when he was in the standing position, in which he had never had the sensation, pressure was exerted about the lower part of the neck and the sensation in the throat was exactly reproduced. Release of the pressure caused it to disappear. Repetition of the procedure when the patient was recumbent caused aggravation of the preëxisting discomfort. Lowering of the head also aggravated the sensation. With the patient in the recumbent position sudden pressure on the abdomen aggravated the sensation. When he was sitting up and entirely free of the discomfort, the same procedure produced it in milder degree. He was told that his discomfort was probably the result of congestion of the esophagus and was advised to be esophagoscoped in order that one might know whether or not varices were present, but he was completely satisfied with the explanation and declined esophagoscopy. Nevertheless, it seems probable that congestion, either with or without large varices at the upper end of the esophagus, was responsible for the rather bizarre symptoms of which he complained.

Insofar as I am aware, discomfort of this type complained of by the patient just described has not been heretofore mentioned in the literature.

Carcinoma of the Esophagus. CASE 4. A man, aged 67, had noted dysphagia without pain for 4 months, and slight pain behind the xiphoid, radiating to the lower substernal region and to the epigastrium, for 3 weeks. The pain was both burning and squeezing in character, was constantly present, and was increased by swallowing, particularly solid food. Physical examination was negative as was the Roentgen ray of the esophagus. However, esophagoscopy revealed a fungating mass which was proved by biopsy to be a carcinoma.

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This patient illustrates the fact that carcinoma may be indetectable except by the esophagoscopic method.

Discussion. As the result of the cases mentioned and of those described by others, certain generalization may be offered concerning chest pain induced by disorders of the esophagus.

Disorders of the esophagus are frequently painless, but in some instances the pain may be of great severity.

Dysphagia is usually present but is absent in many cases. This is particularly true in patients who have peptic ulcer of the esophagus, either with or without esophageal hiatal hernia.[1,2] In such patients pain is an early symptom, while difficulty in swallowing appears late. The reverse is usually the case in subjects with esophagospasm, and in those with carcinoma.

Esophageal pain is likely to consist of a feeling of burning[6] or of fullness, distention or constriction.

The most common location for esophageal pain is the lower substernal region and it not infrequently radiates to the epigastrium, or to the region of the dorsal spine. However, as was pointed out by Moersch and Miller,[8] the pain may be very widespread, may radiate to the ear, to the face, or more commonly to the neck, shoulder, arm and hand. According to the same authors, pain in the forearm and hand is likely to be localized in the radial rather than in the ulnar side. The point may be of some differential diagnostic value with respect to angina pectoris.

The duration of the individual attacks of esophageal pain is very variable. At times the discomfort lasts only a few seconds, while in other instances it endures for weeks.

Among the factors which may precipitate esophageal pain are *swallowing*, which is especially important in patients with spasm, with carcinoma, and in individuals with obstruction due to stricture or to scar tissue as the result of scleroderma; *eating*, which is especially important in patients with peptic ulcer of the esophagus; the *recumbent position*, which is most likely to precipitate the pain in patients with esophageal ulcer (the regurgitation of acid into the ulcerated area is favored by the recumbent posture); the ingestion of *highly seasoned food* and of *alcoholic beverages*, which is likewise especially important in patients with peptic ulcer of the esophagus; and especially the act of *swallowing when the patient is lying down* (here gravity does not aid the descent of the food and any lesion which interferes with peristaltic waves tends to allow food to be trapped when the patient is in the recumbent posture).

Among the factors which tend to alleviate esophageal discomfort are *antispasmodics*—those of the atropine group being the most beneficial; and the assumption of the upright position. The latter factor is especially important in patients with peptic ulcer of the esophagus associated with congenital shortening of this organ and with hiatal hernia. Some patients with this syndrome need to have the head of the bed elevated, because only in this way can the regurgitation of the acid contents from the stomach onto the ulcerated area be prevented.

The character of the pain in the patients reported was sometimes constrictive and sometimes burning. This is in keeping with the observations of Jones,[7] who found that in certain normal subjects burning pain (analogous to "heart burn") was produced by distention of a balloon in the lower esophagus. In other individuals the discomfort consisted of a feeling of pressure. Since the pain of angina pectoris may also be either constrictive or burning,[4] it is evident that the character of the pain is of limited value in differential diagnosis between these two conditions.

The tendency of pain arising from the esophagus to resemble in distribution that arising from the heart, was likewise elucidated by the work of Jones.[7] He noted in healthy persons that the pain produced by distending the esophagus sometimes radiated from the substernal area to the tip of the left shoulder and down the left arm. Furthermore, he observed that distention of the esophagus still caused pain after all of the thoracic sympathetic ganglia had been removed, and concluded that either pain fibers from the esophagus were carried in the vagi, or that some of the fibers passed up the esophagus and entered the cord in the cervical region. Jones pointed out the importance of the factor of summation of afferent impulses from different localities in determining the localization of esophageal pain. Thus, in 1 of his patients the removal of a cervical rib abolished the radiation of the pain to the left shoulder and arm but did not abolish the substernal distress. Similarly, it is possible that the coëxistence of angina pectoris may increase the likelihood of radiation of esophageal pain to the left shoulder and arm, as in the case reported by Eideken, and in the case cited in this communication.

Summary. A series of patients with pain arising in the esophagus and simulating, in some degree, that due to disease of the heart has been presented. One of the patients had both angina pectoris and severe pain due to spasm of the cardiac end of the esophagus.

The location, quality and intensity of the pain due to esophageal disorders may be indistinguishable from that due to disease of the coronary arteries. In both conditions the pain may be precipitated by emotional disturbances and by the ingestion of large quantities of food.

In the differentiation of angina pectoris from pain brought about by disorders of the esophagus the following points are of value.

1. Pain arising from the esophagus is not ordinarily related to muscular exertion.

2. Pain precipitated by swallowing or coming on during eating is likely to be due to esophageal disorders. The ingestion of highly seasoned food or of alcoholic beverages is especially apt to precipitate esophageal pain.

3. The presence of even a minor degree of dysphagia constitutes strong evidence in favor of esophageal pain.

4. The duration of esophageal pain is more variable (a few seconds to many hours) than is that of angina pectoris, which usually lasts for a few minutes.

5. Electrocardiographic, radiologic and esophagoscopic studies are

of great value when they yield positive results, but many errors are made by placing undue emphasis on borderline findings.

6. Atropine and allied drugs often produce striking relief in patients with esophageal pain but do not usually affect anginal pain. Nitroglycerin may relieve esophageal pain but the effect is rarely as striking as in anginal pain.

7. Fluoroscopic study of the esophagus, made while the pain is present, may yield positive results when the same procedure is quite negative, if carried out in the pain-free state.

8. One of the most useful methods of diagnosis is induction of the pain when the patient is under observation. This can nearly always be done by muscular exertion in patients with angina pectoris. Esophageal pain can be precipitated in some patients by the ingestion of highly seasoned food or of alcoholic liquors, and especially if these irritants are swallowed when the patient is recumbent. In other patients esophageal discomfort may be induced by distending the stomach or the esophagus with air or with water. Since these procedures may cause discomfort in healthy persons they are only significant when they reproduce exactly the spontaneous discomfort of which the patient complains.

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CLINICAL ASPECTS OF PAIN IN THE CHEST

III. Pain Arising From the Stomach

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Although the importance of disorders of the stomach and cardiac end of the esophagus as causes of mild distress localized in the lower substernal area is well known, comparatively little attention has been

paid to the significance of such disorders as causes of widespread and severe pain resembling in character and in distribution that often induced by diseases of the coronary arteries. The recent study by Balchum and Weaver[1] in dogs indicated that the sensory nerves of the stomach are derived in the main from the lower dorsal segments but that branches may come from as high as the fourth dorsal level. These observations would account for the occurrence of pain in the middle of the chest. However, the observations of von Bergmann,[3] Jones,[13] and others, as well as those to be reported in the present study, indicate clearly that pain produced by disorders of the stomach may be felt in the upper chest, neck, shoulder and arm, the left side of the body being involved more frequently than the right. Whether pain of this distribution is transmitted by nerves from the stomach itself, or by pathways from contiguous structures—such as the diaphragm, esophagus and heart—need not be considered in this communication, which is not concerned with the route of transmission but rather with the qualities of the pain and with the criteria whereby it may be differentiated from that due to disease of the coronary arteries.

The patients to be discussed represent a selected group. They were seen by the author because they were considered, either by themselves or by their physicians, to be suffering from pain arising in the heart. The majority of patients with gastric disturbances have their discomfort in the upper abdomen and are not likely to present themselves as "heart cases." However, since it is in the exceptional patient, with pain referred to the chest, that confusion is apt to arise, it seems worthwhile to report such a group of selected cases even though they do not present the typical symptoms of their underlying gastric disorders.

"Functional" Distention of the Stomach as a Cause of Pain in the Chest. Under this general category may be grouped the patients in whom the pain seems to be related either to aerophagia, to pylorospasm, or to an unusually low position of the stomach. Since these conditions frequently occur together they need not be discussed separately.

Of the 13 patients in this group, 9 were females. The ages ranged from 30 to 68.

The chief sites of *location* of the pain were in the left lower chest, anteriorly, below the cardiac region (7 patients), and in the precordial area (4 subjects). The remaining 2 patients complained of pain behind the lower sternum. The discomfort radiated to the left shoulder in 3 subjects, to the left arm in 2, and to the back of the chest in 4.

The *duration* of discomfort was variable but most of the patients stated that it lasted more than an hour. The *severity* varied from minimal to moderate and no patient complained of marked distress. Most of them were concerned about the possible significance of the pain rather than about the discomfort itself. The pain consisted of a feeling of fullness, tightness or pressure in 8 subjects, was of burning character in 2, stabbing or shooting in 2, and aching in 1 instance.

The pain was related to posture in 6 subjects, being aggravated by

lying on the left side in 4 patients, was worse on the right side in 1 person and was increased by the upright position in another.

Eleven patients stated that the pain bore no relation to exertion. Two patients gave a history of pain made worse by walking or by other bodily effort. Both of these subjects were subjected to severe exertion under observation and in neither instance did the pain develop.

The relationship of the pain to digestive functions was striking and was the most important feature in the history. Nine of the 13 patients obtained complete relief by belching. Two obtained partial relief and 2 were not affected by this act. Four subjects had noted that expulsion of the flatus gave partial to complete relief from the discomfort, and 3 patients had obtained relief from pain by vomiting. Eating aggravated or precipitated the pain in 4 subjects but produced relief in 1. Two subjects complained of some pain caused by swallowing and it is probable they had cardiospasm in addition to pylorospasm.

There were 3 patients who stated that the pain was definitely aggravated by deep breathing or by cough, and in 2 of these coughing under observation did cause mild pain. These patients were thought at first to have discomfort of pleural origin, but further studies showed that in spite of the relation to breathing and the lancinating character of the pain, the discomfort was arising from the stomach.

Emotional disturbances tended to induce the pain in 6 of the patients. Mild to severe anxiety states were observed in 10 of the 13 subjects, 9 individuals being frightened about the possibility of cardiac disease, and the tenth having cancerophobia. In 7 of the patients the attacks of pain tended to be associated with palpitation.

Positive findings on physical examination were conspicuously absent in this series of patients. Attempts to reproduce the pain by abdominal pressure was successful in only 2 instances, and 4 other patients had minimal to moderate tenderness in the epigastrium.

The results of radiologic examination were likewise not impressive. Pylorospasm could be demonstrated in 5 subjects. Two patients displayed an unusual degree of ptosis of the stomach. One exhibited regurgitation of the barium meal into the esophagus. In the remaining subjects fluoroscopic examination yielded entirely negative results.

Normal electrocardiograms were encountered in 10 patients, 2 subjects displayed frequent ventricular premature beats, and the remaining individual had well-marked inversion of the T waves in the fourth lead. This patient had been incorrectly diagnosed as having angina pectoris, and since his case is fairly typical of the syndrome as it occurs in the older age group it may be summarized here:

A 60 year old male complained of mild discomfort in the region of the left lower anterior part of the chest. The pain lasted from a few minutes to several hours and usually radiated to the left shoulder. It consisted of a feeling of tightness or of fullness, and had been present for about 2 months. When asked about the relationship of the pain to effort he at first stated that it was likely to come on during exertion. It was likewise aggravated by eating large meals but tended to be relieved completely either by belching or by expulsion of flatus. Physical examination revealed questionable cardiac enlargement and slight hypertension, the systolic and diastolic pressures being

170 and 105 mm. of mercury, respectively. There was slight slurring of the QRS complexes in Leads 2 and 3, and T_4 was inverted. The heart was slightly enlarged by Roentgen ray and fluoroscopic examination of the stomach yielded negative results. He had been told that he had angina pectoris and had developed an anxiety state in regard to his heart.

The story that the pain frequently endured for more than 1 hour and that it was so strikingly relieved by belching, plus the observation that the patient tended to swallow air, led to further investigation. On repeated careful questioning he stated that he was not certain whether or not the pain was related to effort but felt quite sure that it tended to come on after a heavy meal. When he climbed stairs under observation (200 steps in 10 minutes) he became very fatigued but had no pain whatsoever. An electrocardiogram taken immediately after the exercise revealed that the previously inverted T_4 had now become upright. When a tube was passed and 1500 cc. of air were slowly introduced into the stomach a feeling of discomfort identical with that which occurred during his spontaneous attacks was induced.

Comment. In this patient the presence of hypertension and of slight cardiac enlargement, plus a hastily taken initial history and overemphasis on the importance of electrocardiographic changes, led to a false diagnosis of angina pectoris, when actually the patient's discomfort was due to a much more benign disorder.

As illustrated in the patient just cited, the reproduction of the pain is at times a valuable procedure in diagnosis. Eight patients in the group were tested in this manner by having their stomachs inflated. Obviously, the introduction of sufficient air or water into the stomach will cause discomfort in anyone. The point of the test was not whether some discomfort was produced but whether discomfort simulating exactly that which the patient experienced during the spontaneous attacks could be induced by this procedure. In 6 of the 8 subjects the inflation of the stomach with air yielded a positive result in the sense of causing pain similar to that of which the patient originally complained. In 1 patient the introduction of air induced discomfort somewhat like that of the spontaneous pain but the patient was uncertain until the air had been withdrawn and 2000 cc. of water was introduced in the tube. She then felt sure that her pain had been reproduced. In the remaining subject these procedures induced discomfort which was quite unlike that which brought the patient to the physician.

The effect of atropine on the pain was studied under observation in the hospital in 7 of the 13 subjects. Five patients obtained either complete or practically complete relief. The remaining 2 subjects were not affected.

As an example of a patient illustrating most of the features of the syndrome of chest pain due to distention of the stomach, the following subject may be cited:

A 36 year old female had suffered for 2 years from attacks of mild burning and aching pain in the left lower chest anteriorly, and also in the lower substernal region. The attacks lasted only a few minutes and usually began at night, especially when she was lying on the left side. The pain was partially or completely relieved by turning on the right side, but was increased in intensity by sitting up or by standing. During most of the attacks of pain she was conscious of pounding and skipping of her heart. Her discomfort was sometimes benefited by drinking cold water, by eating a small amount of food, by the ingestion of soda, or by the expulsion of flatus, while belching

gave complete relief. One of her cousins had had cardiac disease and the patient was afraid that she herself had developed it, and displayed a mild anxiety reaction. The physical examination was negative except for her slender habitus. She was 5 feet 7 inches tall and weighed 101 pounds. Electrocardiograms revealed no abnormality. Fluoroscopic examination of the stomach showed that this organ was very ptotic and emptied more readily with the patient lying on the right side than when she lay on her back or on the left side. When a tube was introduced and the stomach inflated either with water or with air, ventricular premature beats set in and she developed her typical pain. Under observation the pain was aggravated by lying on the left side, or by sitting, and was improved by turning on the right side. Her physical discomfort was completely alleviated by atropine and her anxiety by assurance.

Comment. This patient evidently had some trouble in emptying her stomach either in the upright position or when she lay on the left side, because it occupied a very low position, and in these postures the pylorus was well above the dependent part of the greater curvature. Her discomfort was referred to the chest and thus led to an anxiety state because of fear concerning cardiac disease which did not exist.

The chief clinical manifestations exhibited by patients who have chest pain as the result of gastric distention due to functional disorders may be summarized as follows: In most instances pylorospasm, aerophagia, gastroptosis or a combination of these minor disturbances is present. Although the patient may be of either sex and of any age, most of the individuals with this syndrome are middle-aged females. Anxiety and palpitation are commonly associated symptoms. The discomfort often sets in when the patient is lying on the left side and it usually consists of a feeling of fullness which is likely to be located in the left lower chest, anteriorly, but which may have the typical distribution of pain due to coronary disease. The duration is more variable and is often longer than in the case of attacks of angina pectoris. The pain is usually mild but this fact is of little differential value because pain due to coronary disease is likewise frequently mild. Emotional disturbances and eating often induce the discomfort, which may be relieved by expulsion of flatus, or by vomiting. However, the act of belching and the administration of atropine produce the most striking benefit. The reproduction of the pain as to location, quality, and so forth, by inflation of the stomach, is a valuable diagnostic aid. Pain due to this cause may be stabbing in character and aggravated by breathing, and may therefore offer a superficial resemblance to that due to disorders of the pleura.

Esophageal Hiatal Hernia. Following von Bergmann's[3] studies on the frequency and the importance of this condition as a cause of pain in the chest the clinical manifestations have been discussed by a number of authors[7,14,17,19,25] and more particularly by Jones,[13] who reviewed the symptomatology in 128 cases. He discussed in detail the points of similarity between pain due to coronary disease and that due to hiatal hernia, and pointed out that the smaller herniæ were more likely than the larger ones to produce pain resembling that of angina pectoris. Recently Schnepp[24] has emphasized the importance of hiatal hernia as a cause of pain behind the lower sternum ("heart burn") during the last trimester of pregnancy.

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Of the 24 patients in the present series with thoracic pain due to disorders of the stomach, 4 had esophageal hiatal herniæ. The symptoms in these patients were very similar to those described by Jones,[13] the pain being felt in the substernal region or the lower left anterior chest and radiating to the left shoulder or to both shoulders. The duration of the discomfort was very variable, lasting from a few minutes to as long as 3 days. The pain was mild to moderately severe and consisted of a sense of fullness or constriction. Two of the 4 patients stated that the distress was precipitated by the recumbent posture. All of them noticed aggravation following large meals, and were sometimes relieved by belching. One patient obtained relief by alkalies. (This led to the suspicion of an ulcer either in the hernial sac or in the esophagus but this could not be demonstrated by Roentgen ray and esophagoscopic examination was not done.) There was a tendency for the pain to be aggravated by emotion and by alcoholic liquors. One of the 4 patients also had typical angina pectoris on effort with a slight difference in the distribution and quality of the pain and with relief by nitroglycerin. The pain due to the hiatal hernia was not affected by nitroglycerin.

The only patient in the series presenting symptoms strikingly different from those observed in many of the cases reported in the literature was an individual who apparently developed a perforation of the hernia.

A 57 year old man had had occasional mild sensations of distention and fullness in the left lower anterior chest below the precordium for about 15 years. This usually followed large meals and was relieved by belching. Three weeks before admission to the hospital the attacks began to increase in frequency, intensity and duration. A few days later he noted that in addition to his previous distress he was having a sharp stabbing pain beginning in the same general region but radiating to both shoulders and markedly aggravated by deep inspiration, coughing or sneezing. About the same time he began to have fever and irregular chills and developed an increasing leukocytosis. Physical examination was essentially negative and electrocardiograms showed no significant changes. Fluorosopic study revealed a small esophageal hiatal hernia and upward displacement of the right side of the diaphragm. It was believed that he had a subphrenic abscess and operation was advised. This was refused and after a stormy course he gradually recovered following symptomatic treatment and the use of sulfonamide drugs.

Comment. It seems highly probable, although unproven, that this man had a perforation of an esophageal hiatal hernia, leading to a subphrenic abscess.

Cascade Deformity of the Stomach. This disorder resembles hiatal hernia in that it is a frequent finding in patients who have no symptoms. The typical deformity which was first described by Rieder[21] in 1910 tends to occur intermittently and may be observed to disappear during fluoroscopic examination. The upper pouch is usually situated posteriorly but may be located either to the right or left of the body of the stomach. Hence, the pain when present is likely to be related to position, because drainage of the abnormal pouch usually occurs better in one posture than in another. The discomfort is likely to be completely or partially relieved by lying down. In most of the patients the optimal position is lying on the left side,[6,26] but in some patients

comfort is best achieved by the right lateral posture—as in the case described below—or by the prone position.[21]

The cascade deformity of the stomach usually produces no symptoms. When pain occurs it is usually situated in the left hypochondrium or the epigastrium, as in the cases cited by Upham,[26] by Brown and McHardy,[6] and by Rendich and Connors.[22] The latter authors mention palpitation as a common symptom but little attention has been paid to the fact that in exceptional instances the pain may be localized in the chest and may resemble closely the discomfort produced by diseases of the coronary arteries. Retrosternal pain in 1 patient was described by Schaffner and Burton,[23] while Kaufman[16] and Harrison[11] have described patients with pain in the chest resembling that produced by cardiac disease.

Among the factors which may precipitate pain certain positions, and more particularly the standing or sitting postures, have been mentioned. Other precipitating factors which are operative in certain cases are rapid eating (case of Schaffner and Burton[23]) and swallowing (case cited below). Among the alleviating factors are belching, the expulsion of flatus, bowel movements, and one or the other of the recumbent postures. The relief experienced by measures which empty the large bowel can probably be ascribed to the importance of pressure from a distended splenic flexure in producing the cascade deformity.[8,21] One interesting feature of the condition is that the deformity may be noted when a small amount of barium is swallowed and then disappear when additional barium is ingested and the stomach becomes filled[8] and that, similarly, the pain has a tendency to come on after small amounts of food, and may disappear when additional food is taken.[21]

I have observed 3 patients with pain in the chest due to cascade deformity of the stomach. One of them has been reported elsewhere.[11] The 3 patients were all males addicted to overindulgence in alcoholic beverages. The pain was felt in any part of the left anterior chest and radiated to the left arm. It endured from a few minutes to 2 hours with a distinct tendency toward periods of aggravation lasting from a few seconds to a minute, followed by periods of relative freedom from discomfort of a minute or more. The intensity varied from mild to extremely severe. The pain was cramping in character and tended to be precipitated by bending forward or by lying on the side. Turning to the opposite side produced partial but not complete relief. One of the 3 patients had mild burning pain on swallowing, but the other 2 did not. All of the 3 subjects were emotionally unstable and heavy drinkers, the ingestion of alcoholic liquors tending to precipitate attacks of pain. Of the 3 patients, the 1 presenting the most puzzling and dramatic clinical picture was the following:

A white male, aged 58, had indulged heavily in alcoholic beverages for a number of years. Recently he had abandoned whisky but had taken large quantities of beer, averaging 10 to 20 bottles per day. Ten years before he had had symptoms suggestive of peptic ulcer and a deformity of the lesser curvature of the stomach just above the pylorus had been demonstrated by fluoroscopic examination. He was first seen by the author in a state of mild

congestive failure which rapidly subsided on treatment. At this time a story of mild aching pain in the precordial region and left arm, induced by walking and lasting only a few minutes, was obtained. The heart was slightly enlarged; the systolic and diastolic blood pressures were 170 and 102 mm. of mercury, respectively. Electrocardiogram revealed only left axis deviation with low voltage of T_2 and T_3. Under observation walking a distance of 200 yards produced the discomfort in the chest and arm. The same exertion undertaken at the same rate 2 minutes after the sublingual administration of nitroglycerin caused no discomfort.

Three months later he began to have violent attacks of "squeezing" pain radiating from the substernal and precordial regions to the left pectoral area, and into the left shoulder and arm. These attacks would last from 1 to 3 hours. Paroxysms enduring for about a minute, during which the pain was violent, were separated by 1 or 2 minutes of partial relief. Large doses of opiates were required, and even then he did not obtain complete relief. At times he noted that swallowing caused a mild burning retrosternal pain in the midsternal region. Physical examination revealed moderate cardiac enlargement and slight hypertension, with a tendency for the blood pressure to rise during the paroxysms of pain. Electrocardiograms were taken both during and between attacks and revealed only left axis deviation and isoelectric T waves in the second and third leads. The records taken during the attacks showed no significant difference from those taken when he was free of pain. During the first 4 weeks of illness the body temperature varied from 100 to 101° F., and the leukocyte count ranged from 7000 to 14,000.

The patient was at first thought to have myocardial infarction but as the illness continued with almost daily repeated paroxysms of pain, further investigations were made. Fluoroscopic examination carried out when the pain was absent revealed a typical cascade deformity of the stomach. The barium passed freely through the esophagus into a fundic pouch. Only when this pouch was filled did the barium spill over into the body of the stomach.

On the following day fluoroscopy during a seizure of pain showed obstruction of the distal end of the esophagus and the barium slowly trickled through the cardia into the main portion of the stomach. The fundic pouch could not be visualized in any position. Apparently there was torsion of the portion connecting this sac to the remainder of the organ.

Following this observation the patient was given frequent feedings of a bland diet and large doses of antispasmodic drugs. The pain was practically completely relieved within 48 hours and no further paroxysms occurred.

Comment. In this patient the resemblance of the pain to that of myocardial infarction was at first very striking. The exact mechanism of the pain was somewhat obscure but it seems likely that the proximal pouch became twisted in some way and precipitated the violent attacks of constrictive pain. The possibility that the coëxistence of coronary disease may have influenced the distribution of the pain of gastric origin will be discussed later.

Miscellaneous Disorders of the Stomach. *Diverticulum.* I have observed only 1 patient in whom this disorder caused chest pain and this case had been reported elsewhere.[11] The pain was severe and the clinical picture was dramatic, resembling somewhat that of the patient just described who had a cascade deformity of the stomach. However, the patient with the diverticulum had had recurrent attacks for many years and an erroneous diagnosis of angina pectoris had been made.

Severe chest pain with radiation into the neck, shoulder or arm is quite exceptional in patients with gastric diverticula. However, mild substernal burning or pressure, *i. e.*, pain resembling that of angina pectoris in location and quality (but not in the precipitating factors) is not unusual. Discomfort of this type occurred in several of the cases reported by Bonham,[5] Reich[18] and Reineke.[20]

Trauma to the Stomach. CASE 1. A white male, 48 years of age, 15 years before had fallen on a sharp surveying pin which had penetrated the abdominal wall and stomach which were immediately repaired by operation. During the next year he had frequent seizures of pain in the left side of the neck induced by eating. These gradually became less frequent but he began to have attacks of a milder "pressing" pain in the left lower chest, anteriorly, in the precordial area. The discomfort was partially relieved by turning to the right side, by sitting, and especially by belching. Physical examination and electrocardiograms revealed no abnormalities. Fluoroscopic examination was negative except for aerophagia. Attempts were made to reassure the patient but he insisted that he had "heart trouble" and attempts to convince him otherwise were unavailing.

Postoperative Obstruction. CASE 2. A female, aged 42, had a subtotal gastric resection for peptic ulcer, gastro-enterostomy being performed. Ten days later she began to have severe cramping pains in the left lower chest, anteriorly, radiating to the left side of the neck and into the proximal part of the left arm. The pain was aggravated but not induced by deep breathing and was usually relieved by vomiting. At first the attacks were induced by ingesting liquids. A few days later she could take water comfortably but had pain upon eating small quantities of any solid food. Fluoroscopic examination displayed obstruction of the artificial opening between the stomach and the jejunum. Atropine decreased the frequency and the severity of the attacks. As time passed she gradually improved and about 6 weeks after the operation the pain disappeared entirely.

Comment. It is uncertain whether the pain in this patient was due to edema or to spasm of smooth muscle at the site of the gastro-enteroscopy. The prompt beneficial effect of atropine suggests the latter mechanism.

"Acute Indigestion." CASE 3. A 28 year old white male had been drinking excessively for 48 hours and toward the end of the spree ate a large meal consisting chiefly of barbecued meat. Six hours later he was awakened with a violent squeezing pain, radiating into the neck and to both shoulders. The pain would last for a few seconds and then improve for perhaps a minute and then become more intense again. It was aggravated by breathing. He vomited 4 times during the next hour but this was followed by only temporary relief. He was then brought to the hospital where he was found to be in a state of collapse, the systolic and diastolic blood pressures being 85 and 70 mm. of mercury, respectively, while the heart rate was 45 beats per minute. In spite of his youth myocardial infarction was suspected and an electrocardiogram was made which revealed about 1.5 mm. elevation of the S–T segment in Lead 2, with a diphasic T_3. After the pain had endured for 5 hours, improvement set in and within 16 hours after the onset of symptoms he felt completely well. However, the electrocardiographic changes persisted. When he was questioned carefully it was learned that a year before he had been in an automobile accident and had fractured several ribs in the region of the left axilla and precordium.

Comment. It seems clear that in this patient the acute episode of pain was the result of irritation of the stomach, resulting from alcohol and from the injudicious intake of food. The bradycardia, acute hypotension and clinical appearance of collapse were brought about by the vagal stimulation which often accompanies acute gastric disturbances. The misleading electrocardiographic changes were probably due to an old scar on the surface of the heart brought about by contusion at the time of the automobile accident, as described by Beck.[2]

Discussion. Practically all of these patients who had chest pain arising from disorders of the stomach believed that they were suffering from cardiac disease. In some instances this conclusion had been drawn by the patient himself while in other cases the diagnosis had been made by his physician. Although in some of the patients it was clear

from the outset that no disorder of the heart existed, in others, and particularly in those with the various deformities of the stomach, the differentiation from angina pectoris or from myocardial infarction was very difficult. Among the points which have been helpful in making such a distinction in this series of patients are the following:

The location of the pain is of relatively little differential value. However, when the point of greatest intensity is in the epigastrium, the left hypochondrium or behind the lower ribs, the stomach is a more likely source than the heart. The reverse is true when the area of greatest severity is in the neck, the precordium or the upper substernal region. Pain behind the lower sternum is a common result of either condition.

The duration of pain from the stomach is more variable than is the pain of angina pectoris, which rarely endures for less than 1 minute or for more than 1 hour.[12]

The quality of the pain is of relatively little value in differentiation because constrictive pain, feeling of pressure, and burning discomfort may occur in either condition. Apparently a dull aching sensation is more commonly of cardiac than of gastric origin.

Since both pain arising in the heart and that produced in the stomach may vary from a barely perceptible discomfort to intense agony, the factor of severity is of practically no diagnostic value. However, the constancy of the severity during a given attack is occasionally a helpful guide. Stomach pain may resemble labor pain in coming in "waves" of increased intensity, separated by short intervals of comparative comfort.

The precipitating factors of the pain are of great diagnostic value but may occasionally be misleading. Thus, while distress regularly produced by effort is a characteristic feature of angina pectoris, it is not found in every case. Under exceptional circumstances pain induced by exertion may occur in patients with disorders of the stomach. Jones[15] reported a patient with pain produced not only by eating but occurring regularly when walking a distance of 50 yards. Following resection of an hiatal hernia the patient was able to walk long distances without discomfort.

The tendency of pain to occur when the stomach is distended, and to be relieved by belching, is a characteristic feature in many patients with disorders of the stomach. However, similar events may occasionally occur in patients with angina pectoris and are probably related to the reflex vasoconstriction of the coronary arteries, which may result from stimuli arising in the stomach.[9,10] However, as a general rule, a pain which can be regularly reproduced by distending the stomach with air is likely to be of gastric origin.

Pain related to swallowing or to breathing is not unusual in patients with gastric disorders and may occasionally occur in subjects with recent myocardial infarction. However, these acts rarely, if ever, induce or aggravate attacks of angina pectoris.

Pain arising in the stomach is frequently influenced by posture and, depending on the nature of the disorder and the position of the

lesion, such pain may be affected either favorably or adversely by almost any position of the body. Typically, the discomfort due to herniation of the stomach through the esophageal hiatus tends to come on in the recumbent position. However, anginal attacks, when they occur at rest, are likewise more apt to appear during recumbency and may be relieved by the upright position.[12] This seems to be the only relationship of angina pain to posture. Hence, a pain which is aggravated by lying down—irrespective of the exact recumbent posture—may arise either from the stomach or from the heart, but a pain which is worse in the left lateral position than the right (or the reverse), or which is aggravated by sitting or standing, is more likely to arise in the stomach than in the heart.

Gastric pain is frequently precipitated by alcoholic liquors and often relieved by the atropine group of drugs which have little effect on cardiac pain. Nitroglycerin may benefit pain arising from the stomach but the effect is not likely to be as striking as in the case of anginal pain.

Obviously the electrocardiogram and the radiologic examination of the stomach may be of the greatest value. However, these methods of study frequently yield negative or inconclusive results and may need to be carried out during the attacks of pain in order to be of maximal diagnostic value. Furthermore, minor abnormalities due to healed or insignificant disorders may be erroneously interpreted. Hence, the common tendency to rely almost entirely on these methods and to make only a superficial inquiry into the clinical qualities of the pain is one of the most common causes of incorrect diagnosis and inadequate treatment.

The differentiation of pain arising in the stomach from that of esophageal origin is often impossible from the history alone. Pain of burning quality may arise from either organ* but is more typically of esophageal origin. Localization of discomfort in the epigastrium or behind the lower ribs on the left is more common when the stomach is at fault, but either organ may give rise to substernal distress or, less commonly, to pain in the precordium, left shoulder, or arm. Although pain arising in the stomach is more likely to be affected by posture, esophageal discomfort is often induced more readily by swallowing when the patient is recumbent than by the same act when the subject is upright. The most valuable differential points are the greater likelihood of dysphagia or of pain on swallowing in patients with esophageal lesions and the tendency for belching to relieve pains arising from the stomach. When esophageal pain is related to the ingestion of food it is likely to occur *during* eating while gastric pain more commonly sets in *after* eating. These several points may be of some value in providing a clue as to the most likely site of the pain, but in the majority of instances radiologic or other special studies are necessary before an accurate decision can be made as to whether the esophagus or the stomach is at fault.

* The recent studies of Wolf *et al.*[27] indicate that burning pain probably does not arise from the gastric mucosa *per se* but that disorders of the stomach may lead to it indirectly by causing regurgitation of the gastric contents into the esophagus.

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Attempts to differentiate from each other the several different disorders of the stomach which may cause pain in the chest are likewise fraught with considerable difficulty. Apparently ulcer and carcinoma of the stomach do not usually need to be considered. Despite their relative frequency neither of the conditions was responsible for pain referred to the chest in any instance in the present series. Most of the cases fell into 1 of 2 groups: (1) functional distention of the stomach due to pylorospasm, aerophagia, ptosis or a combination of these factors, and characterized by mild pain, relief by belching, a tendency for the pain to be aggravated by lying on the left side, the coëxistence of palpitation, pronounced relationship to emotion, the presence of anxiety reactions out of proportion to the intensity of the pain, and the frequent occurrence in young to middle-aged females; or (2) disorders dependent on abnormal pouches in the fundic end of the stomach, including herniæ, diverticula and cascade deformities, and characterized by pain of mild to intense severity, radiating to the left side of the chest, shoulder and arm, spasmodic and intermittent character, aggravation by ingestion of alcoholic beverages, marked relationship of the pain to eating and to the position of the body (the posture of maximum severity varying in different subjects), and a tendency to simulate closely the pain of angina pectoris or of myocardial infarction. The differentiation between the several disorders of the latter group can usually be made only by careful and repeated radiologic examination with observation of the stomach in various positions.

The frequency of the coëxistence in a given patient of pain due to coronary disease, and of pain due to gastric disorders, has been stressed in a previous communication[12] and is well illustrated by the patient with the cascade stomach cited in the present report. It seems probable that in such instances the tendency of the two different pains to be referred to the same general regions is related to the factor of summation as suggested by Jones,[15] and by Boas and Levy.[4] According to this concept the existence of subthreshold afferent stimuli from one region tends to sensitize the corresponding segment of the spinal cord to stimuli reaching it from adjacent segments. Such an hypothesis accounts for the observation by Jones,[15] who found that in a patient with a left cervical rib inflation of a balloon in the esophagus caused pain not only in the substernal area but discomfort in the left shoulder and arm. Deep pressure just above the left clavicle, in the region of the cervical rib, reproduced the pain. Following removal of the cervical rib inflation of the balloon in the esophagus caused discomfort only in the substernal region. Jones likewise pointed out that in the presence of operative abdominal scars pain produced by distention of the esophagus or stomach was frequently referred to the site of the scar. The hypothesis of summation of stimuli accounts for such observations, as well as for the tendency for patients with both coronary disease and gastric disorders to have pain produced by either disorder referred to the same general areas. From a practical point of view it would therefore seem advisable to seek with especial care for coëxisting angina pectoris when a patient has pain of gastric

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origin referred to the left arm, and conversely to suspect coëxisting abdominal disease when the pain of angina pectoris radiates to the abdomen.

Summary. The clinical findings—with special reference to the qualities of the pain—have been analyzed in 24 patients with pain in the chest due to disorders of the stomach. In a number of subjects the pain radiated from the precordial or substernal region to the left shoulder and arm, and also resembled in character and in intensity the distress caused by disease of the coronary arteries. Various distinguishing features between pain of gastric origin and that arising from the heart have been discussed. The diagnostic value of radiologic examination during the attacks of pain and of reproducing the symptoms by distending the stomach with air have been emphasized. The necessity for a meticulous analysis of the patient's story of the pain, with especial attention to the various precipitating, aggravating and alleviating factors has been especially stressed.

Among the cases reported in the study are the following:

1. An individual with perforation of an hiatal hernia.
2. A man with mild angina pectoris who subsequently developed violent pain in the chest and left arm, due to cascade deformity of the stomach.
3. A woman with severe postoperative chest pain due to intermittent obstruction of the gastro-jejunal communication.
4. A patient with genuine "acute indigestion" (due to dietary indiscretion) simulating myocardial infarction.

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PENICILLIN IN THE TREATMENT OF INTRACTABLE BRONCHIAL ASTHMA

A Preliminary Report

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This preliminary report on the treatment of intractable bronchial asthma with penicillin records our observations of 2 patients whose treatment was completed 5 months ago and 4 months ago respectively.

Case Reports. Case 1. Le., a woman 45 years of age, with a strongly allergic family history, who has been severely asthmatic since early childhood. For the past 15 years her asthma had been so severe that she required at least 1 injection of epinephrine every night and on very many occasions many injections both day and night. Tests for allergic sensitivity by the intracutaneous method revealed strongly positive reactions to 42 allergens including several inhalants and many foods. Rigid environmental control including several weeks sojourn in a relatively dust-free, air-conditioned room, together with rigid avoidance of all positive reacting foods, occasioned no improvement in this patient's asthma.

For many years she showed clinical and Roentgen evidences of disease of the maxillary sinuses; but it was extremely difficult to decide whether this was a concomitant part of the allergic picture or primary. During all of these years repeated and almost daily examinations of the chest revealed a profusion of asthmatic râles. The asthmatic state was ever present, but despite this the patient never developed status asthmaticus, and had not become emphysematous. There never had been clinical manifestations of cardiac disease.

She has had at least 4 episodes of pulmonary infection, undoubtedly initiated by bronchial plugging, followed rapidly by atelectasis and pneumonic consolidation. On other occasions the sudden onset of localized pleural pain and fever had prompted the immediate administration of epinephrine which probably had a favorable effect on relieving bronchial obstruction, thus preventing the development of pneumonic consolidation.

In June 1942, this patient came under the care of one of us (R. A. C.) at the Roosevelt Hospital. She was placed in an air-conditioned room and given by intent certain of the essential foods to which she was demonstrably allergic by tests. There was neither relief of asthma nor an exacerbation of symptoms during this time. While in the hospital she was tested by passive transfer (Praussnitz-Küstner technique) to 42 of the substances to which she had reacted by direct intracutaneous test. All of the food allergens failed to give any reaction.

Following discharge from the hospital she remained on an unrestricted diet with neither relief nor aggravation of symptoms. At this time she was given an autogenous vaccine made from the organisms obtained by antral irrigation, but with no effect.

In November 1942, she was readmitted to the Roosevelt Hospital for a bilateral Caldwell-Luc operation which was performed by Dr. R. C. Grove. Following this, the patient experienced the usual transitory improvement for several weeks. Since operation she has been free of upper respiratory symp-

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toms. Culture of the antral tissue removed at operation showed *Staph. albus* predominating and *Strep. viridans*.

Early in June 1944, the asthma continuing unabated, the patient was admitted to the Hospital of the University of Pennsylvania, and it was decided to treat her with penicillin, with Dr. Harrison Flippin collaborating. Naturally, we were fearful of giving penicillin, particularly by the intravenous route to this highly allergic patient, and all the more so because she was very sensitive to house dust. Many such patients are allergic to fungi and molds. We did not wish to combine a local anesthetic with the drug given intramuscularly, because we had some evidence that this patient in the past had been allergic to cocaine and its derivatives. We tested her for allergic sensitivity to penicillin in saline suspension, and after we obtained no reaction, we started treatment with this material. Prior to this, sputum culture showed a non-hemolytic streptococcus, which *in vitro* was found to be moderately penicillin sensitive, and this encouraged us to proceed.

She was given intramuscular injections of penicillin every 3 hours, 100,000 units every 24 hours, until she had received a total of 1,375,000 units. At this point the patient developed a violent urticarial reaction with fever, adenopathy, and swelling of the joints. This reaction which had all of the essential characteristics of serum disease persisted for 6 days. Following this she was relieved of asthma for more than 3 months to the extent that she did not require epinephrine. However, although the patient had no subjective symptoms, frequently repeated examinations of the chest showed almost always the presence of a few sibilant râles. Serum for passive transfer was obtained about 1 month after the urticarial reaction and was negative to the penicillin used in the test. This was not identical with the lot used in treatment.

Early in October 1944, an acute upper respiratory infection occurred, with prompt recurrence of bronchial asthma again requiring frequent administrations of epinephrine. This recurrence was complicated once more by severe localized pleural pain and fever. The immediate use of sulfadiazine in full doses, and frequent administration of epinephrine was followed by relief of pleural pain, with disappearance of frictions over this area, and subsidence of fever in 48 hours. Following this administration of sulfadiazine, she had a mild urticarial reaction, similar to that which she had had on numerous occasions following sulfonamide therapy. Since this time the patient has been free of asthma subjectively, but a few sibilant râles can usually be heard whenever the lungs are examined.

Case 2. Wi., a young woman 26 years of age with a definite family background of allergy. Asthma began at the age of 10 years and occured in periodic attacks at any and all times of the year and only following respiratory infections. Over the years the attacks have become more frequent and more prolonged. When first seen in June 1943, dyspnea, cough and wheeze had been continuous for 4 months. The intracutaneous skin tests were completely negative to all the usual inhalant and food allergens and clinical observation could not confirm any ingested food as a factor. Chest examinations showed typical sibilant râles throughout with a moderate grade of emphysema and with a reduction of vital capacity of 25% after epinephrine injection. The heart was normal. Sputum cultures showed pneumococcus as the predominating organism. Clinical and Roentgen examination of the sinuses disclosed bilateral antral involvement especially marked on the right. Under symptomatic treatment and vaccine therapy there was no improvement, and in June 1944 she reported that her dyspnea had been continuous throughout the previous winter with one "cold" after another. Coughing was severe, especially at night, and sleep was consequently greatly disturbed. Examination at this time confirmed the previous findings of antral occlusion. Right antral irrigation yielded mucopus with occasional eosinophils. *Strep. viridans* was recovered from the culture. Penicillin therapy was employed at the rate of 100,000 units daily (12,500 every 3 hours) for 10 days, ending July 8, 1944. Some improvement was observed during hospitalization. Two

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weeks later she reported no asthma and examination of the chest disclosed no adventitious sounds even on forced respiration. Three months later (October 1944) examination showed occasional sibilant sounds after forced expiration but there was no subjective or objective dyspnea and the vital capacity was within normal limits for her height and weight. To date (Nov. 9, 1944) she has had no return of asthma.

This patient had less severe and less prolonged asthma than Case 1, but it had become persistent and intractable for over 6 months. However, both patients belong in the same category in that infection appeared to be the important etiologic factor.

It is obvious that penicillin should be of no value whatever in the treatment of asthma due to air-borne or ingested substances. It is equally apparent that penicillin can be expected to have no therapeutic effect in those patients who have both extrinsic and intrinsic bronchial asthma, if the extrinsic factors are not controlled.

Many young children of allergic soil who develop bronchial asthma early in life have uncomplicated extrinsic asthma. As they grow through adolescence into adult life, and continue to have asthma, the perpetuation of their disease may be the result of acquired bacterial infection. Many of them, then, have both extrinsic and intrinsic allergic disease. Some exhibit strongly positive reactions to many allergens, as the result of childhood sensitivities, but if these cannot be confirmed by passive transfer they may be of doubtful etiologic significance especially when clinical trial fails to produce any symptomatic response. It is reasonable to believe that this is illustrated by Case I of this report.

Those individuals who develop bronchial asthma in adult life, and particularly those who become asthmatic in the 4th decade of life or later, are usually examples of intrinsic asthma, regardless of whether or not the family history is positive for allergic disease. It is possible that penicillin may be found to be of therapeutic value in this group, particularly if the organisms recovered from the bronchial secretion, or from the upper respiratory tract are sensitive *in vitro* to penicillin.

In the presence of clinically demonstrable infection in the paranasal sinuses, or in the presence of other infective foci having an etiologic relationship to bronchial asthma, it is altogether probable that surgical drainage or eradication of such foci may be necessary in addition to the use of penicillin or of any other therapeutic agent to assure any appreciable duration of remedial effect. This remains for the future to decide. However, since penicillin therapy has been shown to have a favorable effect in certain types of purulent sinusitis it may ultimately be shown to be effective in the control of hyperplastic sinusitis of allergic individuals.

We are well aware of the difficulties of evaluating the therapeutic effect of any procedure in the treatment of bronchial asthma. This disease is characterized by spontaneous remissions. Many patients with continuous asthma are improved by hospitalization even if nothing else is done for them. Many asthmatics have periods of remission of considerable duration after acquiring an intercurrent, febrile infec-

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tion. In 1813, Dr. Nathaniel Chapman,[1] then Professor of Physic of the University of Pennsylvania, told his students that for some years it had been his custom to send his patients with intractable asthma to the Mediterranean Littoral, there to contract malaria and hence be relieved of asthma. The induction of artificial fever in the hands of Miller and Piness,[4] Feinberg and his co-workers,[2] and one of us (S. S. L.) in collaboration with Stewart,[3] produced no such fortuitous result.

While it is possible that the severe urticarial reaction which our Case 1 had was responsible for her remission, it is only fair to say that this patient had had several similar but less severe reactions from sulfonamide therapy without any remission at all.

Comment and Conclusions. Of the 2 patients with intractable continuous bronchial asthma, in Case 1, following treatment, there was complete remission of asthmatic symptoms for almost 4 months, although very frequent examinations of the lungs during this time revealed the almost constant presence of sibilant râles. No such remission had occurred in the 15 previous years. An acute upper respiratory infection produced the expected recurrence of asthma; its subsidence has been followed by subjective relief, although sibilant râles are usually present. In Case 2, 4 months have elapsed with no subjective asthma.

Twenty-five additional patients with intractable asthma have either been treated with penicillin very recently or are under treatment by us at the present time. It is impossible at this time to know what results, if any, will be achieved in this group. But already it has become evident to us that penicillin is not a panacea for all cases of asthma due to infection, for some patients have shown no improvement after penicillin therapy.

There is no reason to believe that penicillin will be of the slightest benefit in extrinsic bronchial asthma. It is possible that this drug may be found to be helpful in treatment of two groups of asthmatics: those with both extrinsic and intrinsic asthma provided the extrinsic factors are properly controlled, and those who only have intrinsic disease.

It is possible that penicillin may be of value in that group of cases in which the bacteria recovered from the sputum or from the upper respiratory tract are shown to be sensitive to penicillin *in vitro*.

It will require the careful and detailed study of many patients by many groups of observers over a considerable period of time to determine whether or not penicillin will yield results of sufficient permanency as to warrant its use in the treatment of intrinsic bronchial asthma.

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PEDIATRICS

UNDER THE CHARGE OF
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THE INTRA-DUODENAL SECRETIONS IN CHILDHOOD

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AN aspirating tube passed into the duodenum when the stomach is empty, during the so-called "interdigestive" period, will usually secure on suction a few cubic centimeters of clear golden secretion having a tendency to foam. Analysis of such fluid by the laboratory gives most helpful information to the pediatrician puzzled by digestive disorders otherwise obscure. This Review is designed to summarize the present state of knowledge concerning the intra-duodenal secretions during infancy and childhood. The current wave of intubation reports has furnished much of the clinical data, but the background of general principles derives of necessity from experiments on animals and adults.

Intubation of the child's duodenum is not difficult, once the technique has been mastered. Good descriptions of the technique have been furnished by Müller,[58] Andersen,[6] and Shwachman, Farber and Maddock.[74] Fluoroscopic control facilitates the procedure. Early workers in the field (Hess,[44] Freudenberg[33]), as well as others more contemporary,[6,66,74] have seemed to prefer a slim single-lumen Nos. 10 or 12 French tube of flexible rubber, though Chenoweth[18a] chose one equally narrow but with 2 lumens (Miller-Abbott[57] type). For intubation of the duodenum itself either variety is satisfactory. The jejunum and ileum can be more readily reached with a double-lumen tube having an inflatable bag at its tip.

The nature of the manipulation utilized to withdraw the duodenal contents makes it well-nigh impossible to be sure that the volumes withdrawn represent the total amount secreted during the observation period. Saliva and gastric contents tend to leak into the duodenum; to forestall this it has been recommended that the contents of the stomach be aspirated independently and simultaneously through a second tube reaching into the stomach.[1] Another suggestion has been to introduce a Miller-Abbott double-lumen tube into the duodenum, with a balloon inflated distal to the ampulla of Vater, in order to block leakage of any of the duodenal contents into the jejunum. This latter procedure sometimes causes unpleasant local pains, and on occasion has caused a constitutional shock-like reaction, most alarming to the bystander.[18b] For that reason, among others, it is wise to have the actual intubation of the child's duodenum

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performed by a physician, who should remain close by the subject until the entire procedure has been completed.

The Duodenal Fluid. Any critical survey of the digestive phenomena which take place in the duodenum should consider that the food materials reaching it have already passed through the mouth, esophagus and stomach, and that they have been masticated and squeezed, exposed to salivary and gastric enzymes, mixed with mucus and hydrochloric acid, and otherwise made ready for digestion by the small bowel. Some of the threads in the complex pattern of physiologic interrelationships linking duodenum and stomach have been untangled and laid clear for inspection;[5,9] others remain to be traced. One should also remember that the chymous food released by the stomach flows through the duodenum with great rapidity. Only on chance occasions do Roentgen rays after a barium feeding succeed in visualizing the full contour of the duodenum. The secretions may blend with the food while passing through, but the chemical interactions take place further down.

The duodenal juice may be viewed as a pool or composite of secretions forming simultaneously (1) from Brunner's glands and the duodenal mucous membrane (*succus entericus*); (2) from the pancreas (*amylase, lipase, trypsin*); and (3) from the liver, gall bladder and bile ducts (*bile, mucus*). The relative percentage of components derived from each of these separate sources at any one time depends upon a variety of fluctuating mechanisms, constitutional as well as local. Nervous and hormonal control operate jointly as control mechanisms.[9,96] In addition, the duodenal mucous membrane along with that of the jejunum seems to elaborate several hormonal principles—*secretin, pancreozymin, enterogastrone, enterocrinin, cholecystokinin* and *villikinin*—which play a part in regulating the functioning of other portions of the gastro-intestinal tract. Berger's[12] continuous studies with intubated adults have shown that the fasting duodenal secretion wells out at irregular rhythmic intervals 5 to 50 minutes apart. Pediatric experience is that successive applications of suction at 5 to 15 minute intervals usually secures interdigestive fluid in inconstant amounts before resistance is encountered to the further drawing out of the plunger of the syringe. Specimens contaminated with gastric juice will be paler, more acid, floccule-containing, and of greater volume than those free from such admixture. The reader who desires detailed information concerning recent work with the duodenal enzymes of children in health and disease should refer directly to the contributions of Klumpp and Neale,[50] Andersen,[6] Kajdi and Davison,[48] Farber, Shwachman and Maddock,[26,53,74] and Philipsborn, Lawrence, Gibson and Greengard.[65] He should remember, however, that a number of earlier workers have made pioneer contributions in the same field, notably Hess,[44] Schiff, Eliasberg and Mosse,[73] Davison,[22] Müller,[58] and Freudenberg.[33]

The difficulty in collection is only one of the reasons why examination of the duodenal contents must be but semiquantitative. Each of the component elements which go to make up the interdigestive duodenal fluid seem to spontaneously fluctuate both in potency and rate of secretion. Wide variations in color and in relative proportions of bile acids to pancreatic enzymes are exhibited by the same individuals from hour to hour, suggesting, as has been demonstrated more explicitly with adults, that the rate of formation of duodenal fluid during fasting proceeds erratically and irregularly. Older children tend to produce more potent secretions at faster rates than do infants, and individual differences have been commonly noted.

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Rate of Formation. The rate of appearance of duodenal fluid during *fasting,* as judged by the flow from the tube, varied from 4 to 31 cc. per hour (mean 8 cc.) in Kajdi and Davison's[48] 10 normal infants. The 7 normal infants and young children of Philipsborn *et al.*[65] produced from 8 to 25 cc. per ½ hour in the resting state. Farber *et al.*[26] noted that in infants under 2 months the flow varied from 1 to 5 cc. per hour and was usually near 3 cc.; in infants of 1 year this rate was doubled, and in older children often increased eightfold. The amounts were not affected appreciably by crying or excitement. There was no decrease in *chronic nutritional disturbance,* and a questionable increase in patients with *celiac disease.*

Pancreatic fibrosis seems to be the one condition existent in the pediatric age period in which the fasting duodenal contents are persistently small in volume and strikingly different from the watery contents obtained from all other individuals studied. As Farber[26] describes it, the material is thick and sticky; it adheres to the glass rod or to the side of the test tube and falls very slowly. This gross appearance has a counterpart in the histologic appearance of inspissated material in the acini and ducts of the pancreas.

Stimulation of pancreatic flow, as affected by *humoral* or *neural* mechanisms, becomes reflected in an increase in duodenal fluid. Both in animals and man stimulation with secretin produces a large volume of pancreatic juice rich in bicarbonate but poor in enzymes, whereas parasympathetic (vagal) stimulation evokes a scant flow of thicker juice poor in bicarbonate but rich in enzymes. Comfort and Osterberg[20] have demonstrated both of these effects very clearly for adults, using mecholyl chloride (acetyl-betamethylcholine chloride) as the vagal stimulant.

The effect of *secretin* upon the duodenal fluids of children has been studied by Maddock *et al.*[53] and by Philipsborn *et al.*,[65] who found that secretin may be injected repeatedly into the circulation of infants and children without ill-effects. Maddock noted a secretory augmentation within 3 to 5 minutes, which in normal individuals reached a peak of about double the rate inside of 20 minutes. Six patients with chronic nutritional disturbance and 2 patients with idiopathic celiac disease showed an excellent response to secretin, but the flow was not increased in 7 children with pancreatic fibrosis. Philipsborn's 7 normal subjects also had a doubling of the rate of flow ½ hour after stimulation with intravenous secretin or with intra-duodenally introduced N/10 hydrochloric acid. Results with *mecholyl chloride* in children have not been published.

Specific Gravity. The specific gravity of 10 specimens of fasting duodenal contents collected from 9 infants ranged from 1.002 to 1.010 and had a mean of 1.006 (Kajdi and Davison[48]). These results are essentially identical with the range 1.002 to 1.008 reported for normal adults by Rehfuss and Williams.[68]

Buffer Strength. Freudenberg[33] showed that the buffer substances in the infant's duodenal fluid consist largely of bile acids and their salts, with the glycocholates being more abundant than the taurocholates. In the duodenal juice from a 3 months old infant the total concentration of bile acids was about 0.18 gm. per 100 cc.; while the juice from a 2 year old child contained about 5.5 gm. per 100 cc. He observed that in the pH zone 4.5 to 6.5, 10 cc. of duodenal juice will neutralize 1 to 2 cc. of N/10 hydrochloric acid. From this datum and the known buffer power of cow's milk he then computed that elevating the pH of 100 cc. cow's milk from 4.5 to 6.5 would require some 380 cc. of duodenal juice. Since

cow's milk[23] evokes a more abundant acid secretion on the part of the stomach than does breast milk, the formula-fed infant must elaborate a much more liberal supply of alkaline small intestinal juice than does the breast-fed baby, in order to counteract the greater acidity and greater buffer of cow's milk. Freudenberg attributed the lower duodenal pH values found in intestinal upsets to temporary impairment of the neutralizing duodenal secretions during the acute illness.

pH. From a review of the early literature Freudenberg[33] concluded that the intra-duodenal hydrogen-ion concentration of the *healthy* infant and child when fasting usually falls slightly on the acid side of neutrality, between pH 6 and 7, and that decidedly alkaline reactions are not encountered, even though the enzymatic activities of both crude pancreatin and refined trypsin are at an optimum at pH 8 and 9. Schiff *et al.*,[73] who kept one tube in the stomach to drain away the gastric contents while the tip of another tube was inside the duodenum, found the mean pH in 9 healthy infants during the fasting state (4 to 9 hours after a meal) to lie between 6.8 and 7.6. In 5 healthy infants, using similar precautions against gastric juice admixing, Kajdi and Davison[48] found a mean of 7.8 (extremes 6.8 to 8.2). But in 2 hours after a meal the mean duodenal pH of these latter infants was lower, 5.6 (extremes 5.2 to 7). In over 80 children from 1 month to 12 years of age Klumpp and Neale[50] found that the pH at all ages had a striking tendency to remain fixed in the pH zone 6.5 to 7 in the fasting state, while after a cream meal (pH 6.8) the pH fell in a constant manner to an average slightly above pH 5. Maddock *et al.*[53] found the pH of the duodenal contents of infants and children before stimulation to vary from 6.8 to 8. After stimulation of the pancreas with secretin the pH was increased by approximately 0.5 in all subjects studied except in the patients with pancreatic fibrosis. The rise occurred in 10 to 20 minutes after the injection and persisted in many instances for as long as 40 minutes thereafter.

In the *acute gastro-enteritides* of infancy more acidic conditions prevail. pH values below 4 were noted by Schiff *et al.*[73] and by Müller.[58] Davison[22a] took duodenal readings at postmortem on 11 infants who had succumbed to diarrhea and dysentery, and found the pH to range between 4.5 and 6.2.

A transitory *post-prandial* dip in the curve of intra-duodenal pH was observed in infants by both Schiff *et al.*[73] and by Müller,[58] during experiments in which milk was introduced directly into the stomach by one tube, and then duodenal fluid removed for examination through another indwelling tube at periodic intervals thereafter. The fall in pH was noticeable within 10 minutes, attained its maximum usually in 1 hour, and then slowly rose back to normal in 2 to 3 hours. With breast milk and most cow milk feeding mixtures the low point on the curve was usually at pH 4.5 to 5.5, always lower than the original pH of the milk when first given. With acidified milks, such as buttermilk, the low point was more often between pH 3.5 and 5.

Evidently the duodenal secretion when "pure" is neutral or slightly alkaline, but when "contaminated" with gastric contents is acidic. The exact pH status seems of minor clinical importance, even though the pancreatic enzymes have their optima for activity at neutrality or on the alkaline side, since these enzymes have but scant time within the lumen of the duodenum to be active. Food and secretions together stream quickly into the jejunum, where the succus entericus there elaborated mixes with and takes control over the pH of the material which comes in from the duodenum.

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Secretions Originating in the Duodenal Wall. The large *Brunner's glands* lie in the duodenal mucosa and submucosa close to the pylorus. With human beings it has not been feasible to dissociate the secretions formed in this first part of the duodenum from those coming from the more distal part, which contains the ampulla of Vater and receives the external secretions of pancreas and liver. With experimental animals, however, it is not difficult to prepare surgical fistulæ which will discharge secretions derived solely from the first part of the duodenum and free from admixture with pancreatic fluid and bile. These fistulæ yield typical mucoid juice at a constant rate (Florey and Harding[29] obtained a steady 1.5 ml. per hour from a dog after food) in the absence of any mechanical or chemical irritation. In this regard this segment of the duodenum differs from other parts of the small intestine, in which, except for small amounts of "periodic" juice, fluid will be produced only after local stimulation. According to Florey, Wright and Jennings,[30] who surveyed the reported experiences in animals, this secretion is colorless or faintly yellow and clear, with specific gravity of about 1.008. Centrifuging occasionally gives an inappreciable deposit made up of cell débris and grayish mucin, derived probably from the goblet cells of the villi. The pH of specimens from most animals lies between 8.2 and 8.9 when collected in contact with air, with buffering power dependent mainly on bicarbonate. The content of total solids varies from 1.2 to 1.5%, with slightly more than half of the solids being inorganic in nature. This part of the duodenum reacts to secretin, and has a sensitive acetylcholine mechanism. Pilocarpine stimulates the flow of juice. The alkaline buffer capacity and the high mucin content of duodenal juice serve to protect the delicate villi of the first part of the intestine from damage by the acid stomach contents. It is not possible to say precisely what are the contributions of the Brunner's glands on the one hand, and of the villi and crypts of Lieberkühn on the other, though Brunner's glands may reasonably be held responsible for much of the duodenal secretions, especially the mucin.

Activation of the proteolytic factor in fresh pancreatic extracts by the juice of the small intestine is commonly explained in terms of a *kinase* or *enterokinase* contained in these juices. According to Wright, Jennings, Florey and Lium,[96] animal experiments demonstrate the presence of only 2 secretory enzymes in the *succus entericus* of the duodenum, namely enterokinase and amylase. According to these investigators the other enzymes there present (proteinase, peptidase, invertase and lipase) are endocellular in origin and originate in desquamated intestinal epithelium, or else have been poured in from the pancreas and other structures higher up in the gastro-intestinal tract. The mucosal amylase is probably weak compared with pancreatic amylase, though no exact figures are available, and therefore not of much significance. The enterokinase, on the other hand, appears to be powerful, and no doubt is of importance in activating the pancreatic juice as it emerges into the intestine. Whether duodenal juice contains more enterokinase than does juice from the rest of the small intestine, as Pavlov[64] believed, has not yet been determined. Wright *et al.*,[96] in a limited number of experiments, failed to find any differences between the digestive properties of secretion from the duodenal wall as compared with secretion from lower levels of the small intestine.

Kunitz and Northrop[51] are the most recent workers to study enterokinase. With crystalline trypsinogen the transformation of trypsinogen into trypsin proceeds as a spontaneous autocatalytic process, catalyzed by but independent of the concentration of enterokinase. With crude pan-

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creatic extracts the activation by enterokinase is much more complicated, since these extracts contain a substance which inhibits trypsin, trypsinogen and chymo-trypsinogen. "Addition of kinase transforms the trypsinogen to trypsin which is partly inactivated by the inhibitor, thus decreasing the rate of the autocatalytic reaction. The free trypsin catalyzes the conversion of trypsinogen to form more trypsin and also catalyzes the conversion of chymo-trypsinogen to chymo-trypsin. If the method of activity determinations used determines both trypsin and chymo-trypsin, as is usually the case, the curves obtained when the activity of the solution is plotted against time are S-shaped but asymmetrical."[61b] Whether enterokinase exerts an activating influence on pancreatic lipase is not yet agreed upon.[9]

Enterokinase itself needs an activator *pro-kinase,* according to Waldschmidt-Leitz and Waldschmidt-Graser.[90] They noted that extirpation of the pancreas is followed by a great diminution in enterokinase activity of the small intestine. Babkin[9] criticizes their experiment as inconclusive.

Exocrine Secretions of the Pancreas. The chief enzymes contained in pancreatic juice are amylase, trypsin and lipase. There are no studies of recent vintage to confirm or contradict the traditional belief that the child's pancreas and duodenum give rise also to lesser enzymes such as lactase, sucrase, maltase and rennin in more than trace amounts. Any stimulus which provokes the appearance of any one of the three major enzymes simultaneously calls forth its two companions, both in animals[39,41] and in man.[4,93] Yet, interestingly, the type of foodstuff predominant in the diet bears some influence upon the relative proportions of these three enzymes. With young rats Grossman, Greengard and Ivy[41] found that a high carbohydrate diet produced pronounced increase in amylase content of the pancreatic secretion with decrease in trypsin, whereas a high protein diet evoked an elevation in trypsin. A high fat intake depressed the amylase without calling out more lipase. A low protein, high fat diet repressed all pancreatic enzymes, but when 1% choline was added to this diet the activity of all enzymes increased again. Michelson[56] has reported for human adults that the enzyme content of the duodenal drainage seemed to adjust itself similarly, within a few days, to similar dietary alterations. However, one unusual child having absent trypsin but normal lipase and amylase has been encountered by Farber, Maddock and Shwachman.[26] Trypsin failed to appear after two attempted stimulations with secretion. This boy, age not stated, "whose clinical picture was one of chronic nutritional disturbance," was the only instance of selective dissociation of this enzymatic triad encountered in some 140 infants and children, well and unwell.

The percentage composition of human pancreatic juice, as given by Fearon[26a] is as follows: organic solutes (lipids and enzymes), 0.6%; sodium, 0.25%; chloride, 0.35%; potassium, calcium, phosphates, sulfates, etc., in trace amounts. Stimulation of pancreatic flow by various factors has already been discussed.

Amylase. Amylases are carbohydrases which attack starches and glycogen. The lack of precise information concerning the internal molecular configuration of these substrates obscures the elucidation of the exact processes whereby they disintegrate when digested by the various animal and plant amylases. Pancreatic extracts are capable of digesting raw starch from corn, wheat and potato, whereas salivary amylase must be placed with cooked starches before its enzymatic activity begins.[15a]

Davison[22c] has elaborated upon the factors which make difficult the

titration of duodenal contents for amylase and trypsin. There is no general agreement as to the best definition of a unit. The viscosimetric method gives differences up to 20% in parallel titrations. Results are contingent also upon the substrates, for no two batches of starch (or gelatin) are exactly alike. End-products of the reactions, chemical accelerators and paralyzers, light and radiation, and differences in equipment alter and distort the activity of the enzyme. Davison[22c] cited four technical pointers made by Dr. D. H. Andersen in personal communications: (1) The position of the collecting tube in the duodenum is important and should be controlled by fluoroscopy. Specimens obtained just beyond the bend in the second portion of the duodenum may be 4 times as active as those collected either near the pylorus or in the terminal duodenum. (2) The time between dilution of the enzymes and its assay is a factor in accuracy, especially for trypsin. Titrations of fresh dilutions and of those allowed to stand for 1 hour or more are usually noticeably different. (3) If the patient is dehydrated, a much longer time is required to obtain the necessary 6 to 8 cc. of duodenal contents, and the enzymic assays sometimes are low. Diarrhea and malnutrition are often associated with a marked reduction in trypsin and amylase. (4) Diet and time after the last meal also influence the findings; fasting contents are more accurate. Davison[22b] noted that admixture of gastric juices greatly increased the amylolytic power of duodenal contents, not merely by stimulating the pancreas, but by some sort of specific accelerating action; with some specimens gastric contents increased the amylase activity eightfold. Acceleration of enzymatic starch hydrolysis by bile has been reported also.[78a] Viscosimetric techniques were used by Davison,[22] Andersen,[6] and Shwachman *et al.*,[74] taking as 1 "unit" the amount of enzyme required to decrease the viscosity of an appropriate starch mixture by 20% in 1 hour. Klumpp and Neale,[50] and Philipsborn *et al.*[65] estimated amylolytic activity in terms of the number of milligrams of dextrose developed in a solution of soluble starch over a given period of time.

Amylase activity is *normally* very low in the neonate, and increases gradually with growth into childhood. Many authors have presented data which portray this *age factor*—those of Klumpp and Neale[50] gathered from 74 normal children may be taken as representative. In round numbers, their mean fasting values for infants 1 to 30 months of age was 0.5 mg. (dextrose); for 3 to 12 months, 0.8 mg.; for 1 to 4 years, 2.5 mg.; for 4 to 6 years, 3 mg.; for six to nine years, 4 mg.; for 9 to 12 years, 3.5 mg. The range in values from which these means were compiled were extremely broad, indicating great spontaneous normal variations. When test meals of cream were given these investigators noted a slight increase in mean amylolytic activity, but the standard deviations of the individual readings were so great that such differences between the observed means cannot be considered significant.

Philipsborn *et al.*,[65] critical of Klumpp and Neale's presentation because "unfortunately values for enzyme activity were expressed without regard for volume," recalculated and reëxpressed those results in terms of activity per unit volume of duodenal juice. (Search through Klumpp and Neale's original report fails to find any statement as to whether or not volume was taken into consideration.) The data when "reëxpressed" by Philipsborn give means of less than 2 gm. dextrose per 100 cc. duodenal juice for the 1st year of life; about 6 gm. for 1st to 4th year; about 10 gm. for 5th through 8th year; and about 8 gm. for 9th to 12th year. Philipsborn's own data on 7 normal cases are in accord with Klumpp and Neale's figures.

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Andersen's[6c] data on 54 normal children of assorted ages bring out the age factor even more vividly. Her mean figures, expressed in viscosimetric units, are as follows: age 0 to 2 months, 4.3 u.; 2 to 6 months, 25.3 u.; 6 to 12 months, 113.9 u.; 1 to 2 years, 177.4 u.; 2 to 5 years, 243.9 u. The standard deviation for each mean value was great, obscuring the diagnostic interpretation of individual low readings. As Andersen says, it would seem that the infant is not prepared to digest much starch before the 3rd or 4th month. Farber *et al.*[26] checked on the enzymatic status of 3 premature infants; 2 had very small amounts of amylase, and the 3rd had none. Philipsborn advised that pancreatic amylase not be considered deficient unless less than 0.5 gm. of dextrose is liberated by 100 cc. of duodenal juice in the first year of life, and less than 2 gm. after 1 year of age. Until a greater body of knowledge has been built up concerning normal values and normal physiologic functionings, a wise procedure in presentation of duodenal enzyme data would be to express them in both (1) units per cc. and (2) units per hour, as was done by Farber *et al.*

Kajdi and Davison[48] presented a 5½ month old infant who had the low average of 184 amylase units per cc. of duodenal contents when ill with *acute diarrhea* and otitis media; 1 month later, when convalescent and nearly well, his average had risen to 619 units. Of Andersen's[6c] 7 infants aged 6 to 12 months having diarrhea, 6 had amylase levels below 50 units; in contrast, of 11 control normal infants of comparable age, all but 1 were above the 50 unit level. With *chronic diarrhea* in children above the age of 6 months Andersen found the concentrations of amylase reduced to one-half to one-third below the normal controls. Andersen noted that 3 infants who presented the clinical picture of *marasmus*, with loose, dry skin, persistent diarrhea, muscular atrophy, severe loss of weight and inanition, showed a marked depression of the level of amylase. All showed enzyme concentrations within the normal range after clinical improvement. Farber *et al.*[26] failed to find impairment of enzymatic activity in 46 patients, aged from a few weeks to 7 years, suffering from various kinds of *chronic nutritional disturbance*.

The concentration of amylase in the duodenal drainage of patients with true *celiac disease* (idiopathic steatorrhea) has always been found to be normal.[6,26,65] It has been Andersen's[6] keen observation that many children diagnosed as celiac disease are not truly ill with classical idiopathic steatorrhea. Instead, as careful study reveals,[7a] these patients have *intolerance to starch* in the diet, give a low level of amylase in the duodenal juice, show starch in the stools with no excess of stool fat, and go through a relatively mild but prolonged clinical course. Low-starch diets—not the low-fat ones required for celiac disease (*vide infra*)—alleviate the symptoms and make for more normal growth.

Lipase.—Pancreatic extracts and pancreatic secretions are highly potent for the hydrolysis of the glycerides of the higher fatty acids, but their effectiveness is weak or negligible with substrates of esters compounded of organic acids with alcohols or carbohydrates. Consequently the term lipase, and not esterase, can be applied appropriately to this pancreatic factor. Wilstätter and Waldschmidt-Leitz,[91] Woodhouse,[95] and Glick and King[36] have shown, interestingly, that lipase extracts of animal pancreas often have very little activity when first made, but on standing in liquid solution or when significant amounts of certain substances are present promptly become actively hydrolytic. Among the activating substances are bile salts, egg albumin, the higher alcohols, boiled lipase solutions, and very small amounts of human and animal serum. These results have

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been interpreted as meaning that the lipase is initially formed in a precursor zymogen state, as *pro-lipase*. However, Rabinowitch and Wynne[67] challenge this interpretation, claiming that pancreatic lipase does not require specific co-enzyme activators, and that the observed "activations" are due mainly to the buffering capacities of the added substances, which operate to shift the pH of the reaction-mixture from pH 3, at which the washed enzyme or pro-lipase is prepared, closer to pH 7, which is the optimum pH for pancreatic lipase activity.

During digestion the fat triglycerides are first emulsified by the bile and then split by the lipase. The fatty acids so liberated promptly go into a water-soluble freely diffusible combination with bile salts and are absorbed by the intestinal mucosa in this complex form.[85] Best and Taylor[15a] point out that not all the digestive lipase comes from the pancreas. In the absence of the pancreas or after ligation of its ducts as much as 50% of ingested fat may undergo hydrolysis by the action of the intestinal juices alone.

Goldstein and Roe[37] have *classified* the various commonly used methods of assaying lipolytic activity as being: (1) titrative, using alkali to measure the amounts of fatty acid liberated upon hydrolysis of a given substrate;[19,92] (2) stalagometric, using the rate of fall of drops of a solution containing enzyme, substrate (such as tributyrin),[71] and buffer; (3) manometric, measuring[70] the volume of CO_2 liberated from $NaHCO_3$ by the butyric acid formed on hydrolysis of tributyrin; and (4) electrometric, measuring the change in pH of a buffered solution as the enzyme acts upon ethyl butyrate[83] to liberate free fatty acid. One might cite in addition the turbidometric method of Craver and Walker,[21] which measures by photoelectric colorimetry the changes in turbidity occurring when serial dilutions of tributyrin are exposed to the enzyme for a 2 hour period. For descriptions of tributyrin methods see Goldstein and Roe,[37] and Free and Myers.[32]

Most of the recent work on pancreatic lipase in children has been based upon the titrative approach. This uses a standardized alkali solution to measure the fatty acids liberated within a standard period of time (usually 1 hour) from a standardized oil emulsion.[50,65] Lipolytic activity of the duodenal contents may be stated simply as the number of cubic centimeters of alkali solution required to neutralize the free fatty acids which form, or more indirectly in terms of calculated Willstätter "units."[6,26,92]

It is very difficult to set up standards for normal values from the reported findings of the different investigators. Not only have each group of workers introduced original modifications and simplifications of technique, but in addition their results are presented in a fashion far from uniform. One simple illustration to bring this out is the comment by Shwachman *et al.*[74] concerning substrates. An emulsion of olive oil prepared in their laboratories gave uniformly lower values than did parallel determinations of the same specimens performed with the commercial olive oil preparation used by Comfort.[20] As Free and Myers[32] emphasized, results of enzymatic analyses are better expressed in terms of comparison with control results gathered in the same laboratory, rather than in absolute figures which convey information difficult to consider away from their contexts. This recommendation is complied with in the paragraphs immediately following.

The accumulation of findings may be summed up by stating that wide variations *normally* occur with infants and children of all ages, and that there are no gradations in lipase activity associated directly with age. The sole exception to this latter statement is the data of Klumpp and Neale[50]

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who found negligible lipase content in infants in the 1st year of life. Two of the 3 premature infants of Farber *et al.*[25] exhibited normal lipase; in the third activity was somewhat reduced. No significant diminution was found by Andersen[6c] in patients with chronic diarrhea or by Farber *et al.*[26] in children with *chronic nutritional disturbance*. The 4 *undernourished* infants of Philipsborn *et al.*[65] had markedly decreased duodenal lipase. This was judged to be a transient pancreatic insufficiency since all subsequently attained satisfactory nutritional status and 1 of them when subjected to a second duodenal study approximately 9 months after the original test had regained normal activity. All authors[6b,11,26,28,66,76,82,94a] have described very low or wholly absent lipase in *cystic fibrosis* of the pancreas. Andersen[6c] states that low lipase figures may occur at times in other disease conditions, such as atresia of the common bile duct; therefore the finding of an absent or very low lipase is not of itself pathognomonic of pancreatic fibrosis.

In true *celiac disease* the dried stools may contain 30 to 60% of lipoid matter (the normal beyond infancy rarely exceeds 20%[45]). Analysis shows this fat to be in the split form of fatty acids and soaps. Hence the fundamental lesion would appear to be an impairment of the absorption of fat through the intestinal wall, and not defective breakdown of fat within the intestinal lumen. Confirmation of this interpretation is given by the many reports of the unimpaired secretion of duodenal lipase by these patients.[6c,10,26,62,63,65,81,82] Indeed, the presence or absence of pancreatic enzymes is the chief clinical diagnostic point for differentiating between celiac disease and cystic fibrosis of the pancreas. In the former disorder the pancreatic enzymes are present; in the latter they are absent, or nearly so. The suggestion has been made[24] that the pancreas normally produces some factor necessary for fat absorption, in addition to lipase, and that in celiac disease the pancreatic secretion is defective in this substance, but no presumptive evidence for the existence of such a hypothetical factor has as yet been brought forward.

Trypsin. Generally speaking, proteases can be divided into 2 classes, proteinases and polypeptidases. It was once thought that the proteinases—among them pepsin, trypsin and chymotrypsin—fragment the large molecules, such as whole proteins, proteoses and the peptones, down to the peptide stage only, and that the peptidases hydrolyze polypeptides and peptides to an ultimate mixture of amino acids. But enzymologists now base their differentiation upon the limited ability of peptidases to break off single amino acids only from one end or the other of the peptide chain, in contrast with the greater power of proteinases to dissolve amino acid linkages anywhere within the proteinous molecules. More recent studies, as summarized by Bergmann and Fruton[13b] and Johnson and Berger,[47] are demonstrating that the breakdown of proteins and protein products by enzymes in aqueous solution consists of a most complex set of processes with innumerable subsidiary equilibrium reactions acting one upon another. Working independently and in combination, co-enzymes, co-substrates and acid and base ions act to influence the directions and rates of the multiple component reactions which go on simultaneously. With synthetic peptide substrates pepsin, trypsin and chymo-trypsin exhibit characteristic differences in the conditions and rates of hydrolysis.[13a] It has been elucidated, chiefly by animal studies, that most of the proteolytic behavior of pancreatic juice is due to proteinases; the peptidases originating in the pancreas seem weak and few in number.

Pancreatic juice, as freshly secreted, will exhibit no proteolytic activity

until after coming in contact with acid or with enterokinase of the small intestine. This means that the proteinases of the pancreas are secreted in a *precursor* or zymogen form, in the same way as the pepsin of the stomach first appears as pepsinogen.

A few of the proteolytic principles in the pancreas have been *isolated* in relatively pure states. Thus, from the pancreas glands of cattle, Northrop and Kunitz[61] have crystallized 2 proteinases and 1 carboxypeptidase. The more potent of these crystalline proteinases is termed trypsin, the other, chymo-trypsin; they seem to be the principal proteinases in pancreatic juice. Neither alone can digest proteins very far, though acting jointly they split most proteins, such as casein, gelatin, edestin, peptone and denatured hemoglobin, down to the polypeptide stage. The existence of a third proteinase, "*hetero-trypsin*," in pancreatic extracts has been hypothesized by Bergmann, Fruton and Pollock.[14] Chymo-trypsin, which has an entirely different crystal formation from trypsin, will hydrolyze casein more completely and at different linkages than does crystalline trypsin. Crystalline trypsinogen and crystalline chymo-trypsinogen are the respective inactive precursors of these two active principles. Chymo-trypsinogen becomes activated by trypsin but does not respond to enterokinase.[80] The conversion of trypsinogen to trypsin is initiated by the enterokinase of the small intestine, and this co-enzyme in its turn is thought to require activation by a prokinase also present in pancreatic juice.[90]

Kunitz and Northrop[51,61] find the transformation of crystalline trypsinogen into crystalline trypsin to be accelerated by the addition of trypsin or enterokinase or by concentrated solutions of magnesium sulfate or ammonium sulfate. When trypsin is added to trypsinogen the reaction follows quite closely that of a simple autocatalytic reaction. The rate of activation depends on the pH and is maximum at pH 7 to 8. The reaction is complicated by the fact that an inert protein is also formed from trypsinogen in the presence of trypsin. The optimum hydrogen-ion concentration for digestion of casein by crude trypsin, crystalline trypsin and crystalline chymo-trypsin was found to spread as a flat maximum from pH 8 to 9. Kunitz and Northrop[51c] studied also the long-known substance in pancreatic extracts which inhibits the action of trypsin. They succeeded in isolating rod-shaped crystals of this inhibitor and hexagonal many-faced crystals of an inhibitor-plus-trypsin compound.

The complex nature of the pancreatic peptidase has been studied in some detail by Abderhalden and Schwab,[1] and Abderhalden and von Ehrenwall,[2] and the presence of a *leucin peptidase*, a *tyrosin peptidase*, and several *acylases*, has been demonstrated. Waldschmidt-Leitz and Kofranyi[88] have described a pancreatic "protaminase" or *carboxypeptidase*, which splits arginine from the carboxyl end of the clupein molecule. A *prolinase* or *prolylpeptidase*,[3] a *carboxypeptidase*[8] and a *dehydropeptidase*[15] have also been reported. The *carboxypeptidase* has been crystallized by Anson[8] and by Northrop.[61b] It seems to be secreted in the form of an inert and as yet unpurified precursor, *pro-carboxypeptidase*, which becomes activated when in contact with enterokinase or with trypsin. Descriptions of the pancreatic proteinases and peptidases, with photographs of the crystals, will be found in the monographs of Northrop[61b] and Tauber.[80]

It is customary to refer to the sum of the protein-digestant principles in pancreatic juice as "trypsin," and that is the meaning of the term as usually employed for clinical purposes, even though Northrop and Kunitz[61a] have applied the term in a more restricted sense to one of the two highly

active proteinases which they were able to extract from animal pancreas in crystalline form.

A diversity of *methods* are available to the investigator desirous of measuring the activity of protease preparations. Tauber[80] speaks of the following general approaches: the increase in amino groups (in the substrate); the increase in the COOH groups; the manometric estimation of amino nitrogen; the Kjeldahl method for non-protein nitrogen; the estimation of residual protein nitrogen; the change in viscosity of protein solutions; milk clotting; the liberation of tyrosine from hemoglobin. In their approach to the trypsin activity of children's duodenal juice Andersen,[6] Shwachman *et al.*,[74] and Kajdi and Davison[48] employed a viscosimeter with gelatin as substrate. Klumpp and Neale,[50] and Philipsborn *et al.*[65] measured the amount of soluble nitrogen liberated from a casein substrate. Andersen and Early[7] recently recommended a modified Fermi method. This is based upon the property of gelatin to no longer solidify at refrigerator temperature after being enzymatically hydrolyzed beyond a certain point. By preparing serial dilutions of duodenal fluid it is possible to measure in roughly quantitative terms the quantity of trypsin contained in a unit volume of duodenal juice. Both Andersen and Early,[7] and Shwachman *et al.*[74] found a close and reliable correlation between results yielded by this simple gelatin-dilution technique and the more elaborate viscosimetric approach.

It may be stated, on the basis of published results, that while one finds wide variation in the trypsin content in fasting secretion among *normal* individuals no significant trend of change has been observed with relation to age.[6,26,48,50,65] Even the 3 premature infants studied by Farber *et al.*[26] had values within normal limits. *Celiac disease* patients showed no significant diminutions in duodenal trypsin.[6,26,65] Infants with *marasmus* exhibited low trypsin activity.[6c,65]

In *cystic fibrosis of the pancreas*, all authors[6c,25a,26,65,94a] described complete or nearly complete absence of duodenal trypsin. Andersen[6c] advised to rely primarily on the assay of trypsin in making a diagnosis of this disorder. She pointed out that duodenal amylase is normally low in early infancy and may be extremely low in the presence of chronic diarrhea without pancreatic fibrosis. Furthermore, the swallowing of saliva, especially when gastric acidity is low, may produce deceptively high results in cases of pancreatic deficiency. For technical reasons the assay of lipase is less reliable than that of the other 2 enzymes. Moreover, low lipase figures have been obtained for a number of patients with other disorders. In no instance, however, in her experience, had trypsin been absent from the duodenal juice of an infant or child without pancreatic deficiency. The low values found in association with extreme marasmus were above any that were found in cases of pancreatic fibrosis, and a second determination after clinical improvement was reassuring. Assay of trypsin in the duodenal juice is therefore the most reliable enzymatic means of diagnosing pancreatic deficiency.

The Bile. In chemical constitution the bile in healthy childhood appears to run more or less parallel to that of the normal adult. Geptner's analyses,[35] cited by May, indicate that in infancy a random specimen of bile will contain (in round numbers): water 93%, mucin 1.5%, sodium glycocholic acid 2%, sodium taurocholic acid 1%, cholesterin and other lipids 2%, mineral salts 0.5%. Sobotka[78a] has commented on the limited worth of quantitative analyses of bile. Great fluctuations occur in the proportion of various constituents with specimens secured in gall bladder

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drainage as well as with human fistula fluid. During and after a period of active hepatic secretion the composition of the gall bladder bile will approach that coming directly from the liver, whereas during stasis the relative content of solids will increase due to absorption of water and salts through the gall bladder wall. It is impossible to obtain reproducibly uniform conditions for the repeated withdrawal of specimens from the same or different individual subjects. Dissimilar methods of analysis also give rise to discordant results, especially with the various pigments and bile acids. For a review of the chemical and biologic literature on bile up to 1937, the reader is referred to Sobotka's[78a] detailed monograph. Agents such as bile salts themselves, which stimulate secretion by the liver, are called *choleretics*, whereas magnesium sulfate and fatty meals which cause emptying of the gall bladder are *cholagogues*. *Anti-choleretics* and *anti-cholagogues*, chiefly pharmacologic substances, exert the opposite effects.

In children, just as with adults, specimens of bile for examination can be readily procured by introducing a tube into the duodenum and injecting a few cc. of a 25% magnesium sulfate solution, or cream, or olive oil. Zelditch *et al.*,[97] Lowenberg and Mitchell,[52] Melamed,[55] Gallerani[34] and other clinical investigators all report that the three "types" of bile—*A*, *B* and *C*—make their appearance in successive order, just as with adults. *A* bile, golden-yellow, is supposed to come from the common duct; *B* bile, darker, more viscous and more abundant, presumably derives from the gall bladder; and *C* bile, clear, light yellow, and of low specific gravity, is assumed to be freshly secreted bile from the liver. Zelditch, Wurmann, Jolkver and Wunditch[97] present the following values for the biliary drainage of children: specific gravity, 1.018 to 1.033; quantity of *B* bile (in ½ hour or before the appearance of *C* bile), 25 to 40 cc.; bilirubin, sufficiently abundant to be detectable up to dilutions from 1:100 to 1:800. They noted that color in bile specimens runs more or less parallel to the quantity of contained bilirubin.

The bile salts and other *surface tension* reducing substances present in the bile aid the digestive processes by adsorption onto the colloidal interfaces of the chyme-enzyme mixture and alteration of the electrostatic equilibria there present. The surface tension (dynamic) of the duodenal contents of 10 fasting infants was found by Kajdi and Davison[48] to be fairly constant, with a mean of 0.459, and extremes of 0.434 to 0.484. These values were determined by the stalagmometer method, and were expressed as ratios relative to water. Sobotka[78a] concluded that bile increases the efficiency of pancreatic lipase, by both maintaining a favorable environmental pH and specifically activating the enzyme itself. With other enzymes such as amylase and trypsin, bile seems to accelerate enzyme action in a non-specific fashion, probably by virtue of its buffer capacity.

With *infants receiving milk* both Schiff *et al.*,[73] and Müller[58] have observed that the bilirubin coloring of the duodenal secretions begins to fade by 15 minutes after the milk is introduced into the stomach, and is usually completely absent 15 minutes after that. Obviously entry of bile into the duodenum has temporarily ceased. Return of color becomes noticeable 1 to 1½ hours after the feeding, more or less coincident with the stage of recovery from the post-prandial dip in intra-duodenal pH. Schiff postulated that the increased acidity evidenced by the lower pH caused a spasm of the sphincter of Oddi with a transient obstruction to the outflow of bile, but Müller was unable to establish any close correlation between

acidity strength within the duodenum and presence or absence of bile therein. The exact mechanism of this non-stimulating or anti-cholagogue phenomenon merits further exploration. It is not impossible that one reason why a meal of milk fails to call forth any bile rests upon the fact that milk curdles in the stomach before being discharged through the pylorus.[94b] In curdled milk the cream globules are trapped within the mesh of coagulum; hence no fat lies free in the whey to mingle with the duodenal secretions and stimulate the outflow of bile.

Zelditch *et al.*[97] suggest that *cholecystitis* is more prevalent than generally appreciated, and recommend biliary drainage for all cases of obscure abdominal pain, whether acute or recurrent. They described 34 instances of cholecystitis encountered at their clinic in Kiev, in children between 6 and 16 years of age. Five were acute, whereas 16 had had symptoms 1 to 4 years. Fourteen were found due to lamblia infestation. All patients exhibited abnormal biliary returns, which established the diagnosis. Except when giardia was present repeated drainages usually brought the bile back to normal, with concomitant relief or amelioration of symptoms. Inflammatory changes—excessive mucus, floccules, leukocytes, parasites and crystals of cholesterol—were usually present in both the *A* and *B* fractions. Only rarely was the *A* bile normal when the *B* bile was inflamed. When the *C* bile was altered also, as happened often, this was interpreted as meaning involvement of the hepatic passages as well as of the gall bladder and its duct. Lowenberg and Mitchell[52] reviewed the subject of childhood cholecystitis. In 1 of their patients biliary drainage was applied with satisfactory results in diagnosis and treatment; in another, the diagnosis was established by this procedure and confirmed later by operation. Melamed[55] described the findings in 32 children aged 3 to 14 who were subjected to duodenal drainage as an approach to the diagnosis of their complaints of abdominal pain. Five were found to have inflammatory changes in the biliary fluid similar to those noted by Zelditch *et al.* and 12 yielded giardia lamblia in abundance. The cases diagnosed as having inflammation of the gall bladder and bile ducts were treated by means of repeated duodenal drainage, using magnesium sulfate solutions and peptone cholagogues, "with excellent results."

Gallerani[34] presented 7 cases of what he termed *catarrhal anicteric angiocholitis.* All patients were girls of pre-puberty age, and all had recurrent upper abdominal pains restricted to or most intense in the upper right hypochondrium. On duodenal drainage their specimens yielded coagulated bile, mucus, cholesterol crystals, epithelial cells and abundant numbers of leukocytes occurring singly and in clumps. Inasmuch as these inflammatory findings were always demonstrable in *C* bile, but not constantly present in the *B* bile, Gallerani felt justified in diagnosing inflammation within the hepatic duct system as the primary cause of the symptoms.

For years it has been known that *Giardia lamblia* may infest the duodenum and upper jejunum in myriad numbers. Yet it is surprising to note how few clinical discussions of giardiasis have appeared, in view of the growing recognition of the pathogenicity of this protozoan and its great susceptibility to atabrine therapy. Smithies,[78] who in 1928 reviewed the subject of giardia infestation, had described how with adults these parasites may give rise to duodenitis, cholecystitis and ulcerative colitis and ileitis. He could usually recover the parasites from the gall bladder when patients who had previously exhibited the parasites in duodenal drainage were subjected to operation.

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Melamed[55] treated 12 giardiasis children with small doses of an acridine derivative (Sostol) for 3 days. All cleared completely. In several of these cases the protozoa were demonstrable on duodenal drainage, yet the stool examinations were negative.

Maris and Bushong[54] reported on some 86 children infested with giardia. All were 12 years of age, 74 being below 9 years (50 males and 36 females). The duodenal juice was examined in 9 of 81 cases having positive stools and always found to be positive; 5 other children showed negative stools, but nevertheless had parasites demonstrable by duodenal intubation. The most common complaints were abdominal pain, anorexia, failure to gain weight, nausea and vomiting. Some 50% had from 2 to 24% eosinophils in the differential white blood count. Most of the cases were cured by treatment with atabrine, in dosages from 45 mg. twice daily for the 2 to 4 year olds, up to 90 mg. thrice daily for those 9 years old and upward, over 3 successive days, followed 48 hours after the last tablet with a small dose of magnesium sulfate. One week after treatment, of the 44 children who reported back, 39 no longer suffered from their original primary complaints. The duodenal contents of the first 27 children were examined after the course of treatment and found free from giardia.

Indirect evidence that the duodenal lipase is normal in giardiasis is given by Véghelyi,[84] who studied the digestion and absorption of fat in 14 children having this condition. With 10 of these children the fat content of the dried feces was greater than 20% of the fat ingested, indicative of impaired absorption of this food material. Interestingly, though, more than 60% of this unabsorbed fat was in the form of free fatty acids (normally only about one-fifth of the excreted fat is in the free form[45]). This high percentage of split fat, coupled with the finding that the actual lipolytic activity of the feces of all the patients was normal when tested enzymologically, was interpreted as meaning that there was no deficiency of fat-splitting ferments in the pancreatic and intestinal secretions.

Internal Secretions of the Duodenum. Physiologists have demonstrated conclusively that properly prepared extracts of the duodenal and jejunal mucosa contain principles which when injected intravenously act to provoke or inhibit functional activities of other portions of the gastrointestinal tract. These principles must reach their receptor parts through the blood stream if they are to be effective; local application or injection are unproductive of results.

Secretin, the first-discovered and best known of these hormones, stimulates the production of fluid and bicarbonate by the pancreatic acini, and enzymes only moderately. Moderate stimulation of bile and intestinal juice is also induced. Purification to the point where a uniform crystalline salt can be isolated—secretin picrolonate—has been announced by Greengard and Ivy.[40]

From the mucosa of the upper small intestine—the same source that yields secretin—of pig, dog and cat, Harper and Roper,[43] and Greengard *et al.*[39] have recovered a substance which when injected intravenously into cats causes a marked increase in the formation of amylase, trypsin and lipase, without augmenting the output of the inorganic constituents. This hormone, which has been named *pancreozymin*, can be separated from secretin by chemical means, and is distinct from it.

The duodenal and upper intestinal mucosa of hogs and other animals contains another specific hormone, *enterogastrone*, which when injected parenterally inhibits the motility and secretion.[38] The secretion becomes reduced not only in volume, but in HCl and pepsin strength as well.[42]

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Cursory mention may be given also to *enterocrinin* (Nasset[60]) which seems to elicit secretion from Brunner's glands and succus entericus from the jejunum and ileum; to *cholecystokinin* (Ivy[46]) which seems to induce gall bladder evacuation; and to *villikinin* (von Kokas and von Ludány[86]) which stimulates movements of the intestinal wall and may be hypofunctioning in celiac disease.

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GYNECOLOGY AND OBSTETRICS

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THE Rh FACTOR IN PREGNANCY

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Among the perplexing problems which face the medical profession is that of transfusion reactions. The giving of blood is no longer a compli-

cated procedure reserved only for the most critical emergencies, but now is administered as soon as a patient's condition warrants such additional supportive therapy. With an increase in the number of transfusions, various types of reactions are being recorded. The type of reaction varies from the mild reaction, where a slight chill and fever occur, to the very severe ones, characterized by chill, fever, hemoglobinuria, jaundice, oliguria, convulsions, and occasionally death.[3,21]

Excluding reactions due to faulty administration, incorrect typing or cross-matching, accidents occur in two groups of patients. One group consists of persons who have received repeated transfusions at short intervals. The other group consists of pregnant women who have developed a reaction on the first transfusion. In this latter group some patients, at subsequent pregnancies, have given birth to jaundiced infants, diagnosed as having erythroblastosis. Although many investigators have studied transfusion reactions for years, it is only recently that any really significant discoveries have been made.

Landsteiner in 1901 described the well-known blood groups designated as A, B, AB, and O. In 1927 Landsteiner and Levine discovered a new series of factors, the M and N factors, which are independent of the above groups. They occur individually or in combination with each other, and assist greatly in the exclusion tests of paternity. No anti-M or anti-N agglutinins normally occur, so they have no importance in the problem of blood transfusions.

It is now accepted that many transfusion reactions occurring in pregnancy or the postpartum period are due to the development of Rh antibodies in Rh negative women who are carrying or have recently carried Rh positive children. It is these Rh positive and Rh negative factors which will be discussed in this paper.

History. The essential facts which have been discovered in the past few years concerning the Rh factor are as follows:

In 1932 Diamond, Blackfan and Baty[7] were the first to suggest that erythroblastosis fetalis could be divided into three separate syndromes consisting of universal edema of the fetus, icterus gravis, and anemia of the newborn. A critical analysis of 20 cases suggested they were all closely related.

In 1940 Landsteiner and Wiener[15] reported a new agglutinable substance, designated as Rh, of human blood unrelated to the other agglutinogens. This property was designated as Rh because it was detected first with the aid of an immune serum prepared by injecting the blood of rhesus monkeys into rabbits. Those individuals whose blood contained the antigen and would react with the rabbit serum were considered Rh positive, while those whose blood failed to react were considered Rh negative.

The Rh factor differs from the M and N factors in that it produces anti-Rh agglutinins, whereas the M and N factors do not produce anti-agglutinins. Therefore it is related to blood transfusions and their various reactions where the M and N factors are not.

Later in 1940, Levine[18] found atypical isoagglutinins in 5 patients. These had the unique property of greater activity at body temperature than at lower temperatures. In 1 patient the agglutinin apparently resulted from the antigenic stimulus of repeated blood transfusions, while in the remaining 4 individuals the reaction accompanied complications of pregnancy such as repeated miscarriages and death of the fetus. It was assumed that in these latter cases the warm isoagglutinins resulted from immunization of the mother by certain dominant, agglutinable substances in the fetus, and that these agglutinable substances are inherited from the father. The agglutinins showed a specificity which differed from

that of the anti-M and anti-N factors, and tests made on the agglutinin from one serum showed it to be similar to that of the anti-Rh factor of Landsteiner and Wiener.

Levine and Katzin[18] in 1940 noted in some pregnant women the development of isoimmunization after transfusion. The sera of these people showed atypical warm agglutinins at 37° C., which corresponded in specificity to the anti-Rh of Levine and Wiener. The antibody acted on the cells of the husband, lending the support to the theory that under certain conditions a mother may be immunized by her fetus.

The work of Landsteiner and Wiener[16] by 1941 led to the belief that the Rh factor was inherited as a simple Mendelian dominant and is not sex-linked. It involves pairs of the allelomorphic genes Rh and rh, with the Rh being dominant over rh (Rh negative). Both the genotypes Rh Rh and Rh rh will be Rh positive.[22,27]

By 1942 Davidsohn and Toharsky[4] found that the Rh factor was composed of several fractions, and that the Rh antigen in most human blood was not identical with that found in the blood of rhesus monkeys.

Race and Taylor[24] in 1943 discovered a factor in the serum of an Rh positive mother of an erythroblastotic infant, which factor reacted with Rh negative blood and with heterozygous Rh positive blood. This was the beginning of the separation of the genotype of Rh negative persons in order to determine whether they were homozygous or heterozygous.

In 1943 Levine[17] found that isoimmunization is induced by other factors in human blood such as A, B and "Hr" (anti-Hr is Levine's designation for an atypical agglutinin seemingly related to the Rh factor, and found in an Rh positive mother who had borne an erythroblastotic infant). The Hr serum is gotten from an Rh positive mother who has had an Rh negative child. This serum is important in two respects. First, it is useful in checking the existence of an Rh negative factor. Second, it helps in the separation of certain gene types in order to determine if the husband is heterozygous. This factor is identical with the agglutinin described by Race and Taylor above.

In 1944 A. S. Wiener[28] carried out tests on a number of patients with erythroblastotic babies where the usual tests for anti-Rh agglutinins were unsuccessful, and in most cases clean-cut blocking reactions were obtained. This proved the presence of a special sort of anti-Rh isoantibody. The reaction is due to an antibody that becomes fixed to the test cells from which the supernatant fluid has been removed before the anti-Rh test serum was added.

Occurrence. The Rh factor is an antigenic substance present in the red blood cells of 85% of the white population of the United States[1] and England.[10] It is absent in 15%, and such individuals are classified as Rh negative. According to Levine, the Rh factor differs in its incidence in the white and colored races. There are 15% of Rh negative white individuals in contrast to only 5 to 8% of colored persons. The condition is rare in the Chinese race. Of 150 Chinese persons tested in New York City, only 1 was found to be Rh negative.[19] Invernizzi[11] states that the percentage of Rh positive factors in South America is "greater in mestizo people, Indians and colored people, than in the white people." The proportion is from 93 to 99% in the former and only 85% in the latter. Landsteiner[14] studied the erythrocytes of 120 full-blooded American Indians and 155 Indians of mixed ancestry for Rh agglutinogens. The blood of only 1 of the 120 full-blooded Indians lacked this factor. In the Indians of mixed parentage the incidence of Rh negative individuals was half way between that found in white persons and in full-blooded Indians.

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The Detection of the Rh Factor. The presence of the Rh factor is determined by placing a suspension of erythrocytes in 0.01 cc. of a 2% isotonic solution of sodium chloride with 0.02 cc. of anti-Rh serum in a test tube for 1 hour in a water bath at a temperature of 37° C. Fresh blood is used since the agglutinating ability of the Rh factor in the erythrocyte is easily lost. A positive reaction is shown macroscopically by the presence of rugosities around the border of the globular sediment in the test tube. This gives the sediment in the bottom of the tube a rough appearance. A negative reaction is indicated by a smooth sediment in the bottom of the test tube. Upon agitation, clumping of the red blood cells is visible grossly; and this finding always should be checked by microscopic examination.[8]

There are five main factors associated with the Rh factor which are universally designated as Rh_1, Rh_2, Rh_0, Rh′, Rh″. The three anti-sera used in all testing are therefore known as anti-Rh′, anti-Rh″, and anti-Rh_0. The cells that are agglutinated specifically by these sera are called respectively Rh′, Rh″, and Rh_0[26].

In the same way as it is possible for agglutinogens to pass from the fetal to the maternal circulation, it is possible also for the Rh antibodies, formed in the maternal blood, to leak back into the fetal circulation and thus produce erythroblastosis fetalis. This condition is characterized by the presence of numerous nucleated red cells in the fetal circulation. The widespread persistence of extramedullary hematopoiesis is the response of the body to an excessive loss of red cells, and the occurrence of immature red cells in the circulation discloses the inability of the body to provide a sufficient number of mature cells. Furthermore the leakage of Rh agglutinins from the maternal into the fetal circulation leads to a continuous destruction of fetal red cells. During neonatal life the agglutinins present at birth continue their work until they are exhausted. If the infant is tided over this period by transfusions of Rh negative blood, the destruction of red cells will cease and its condition will become relatively normal.

Witebsky *et al.*[30] tested red blood cell suspensions from blood clots of 9 individuals of Group O against: (*a*) guinea pig immune serum, (*b*) serum of a mother of an erythroblastotic infant, and (*c*) breast milk from the mother. They found that blood specimens which were Rh positive with guinea pig's immune serum and with the patient's serum were also positive when tested against the milk. They concluded, therefore, that the milk contained the same Rh antibody as the blood serum. In a further study, Witebsky and Heide[29] found Rh antibodies in the breast milk of 2 mothers who recently had delivered erythroblastotic infants.

Erythroblastosis Fetalsis. Macklin[20] defines erythroblastosis fetalis as a condition in which the blood of the fetus exhibits immature cells of the erythrocytic type which are not normal as to either type or quantity for the stage of development of the fetus. She assumes that hemolytic disease of the newborn, as well as erythroblastosis fetalis, is due to other factors than hemolysis. If fetal erythroblastosis is due to an antigen-antibody reaction, hemolysis will occur with accumulation of iron in the fetal liver and with persistent extramedullary hematopoiesis. This is known as hemolytic disease of the newborn and is usually due to the Rh factor complex.

The typical picture of erythroblastosis fetalis is that of jaundice appearing soon after birth, enlargement of the liver and spleen, and other symptoms. These may include varying degrees of any of the following conditions: somnolence, listlessness, and flaccidity; muscular twitchings, opisthotonos, and occasional convulsions; vomiting and diarrhea; petechiæ,

ecchymoses, bleeding from mucous surfaces, increased bleeding and coagulation times; edema, cyanosis, and respiratory difficulties; and collapse. In addition, a large number of circulating immature red blood cells (normoblasts and erythroblasts), a leukocytosis, an eosinophilia, or a monocytosis may be present. The hemoglobin percentage and number of red cells are reduced, but the color index is above normal.

The child may appear strong and vigorous at birth, but symptoms may appear very soon. Death may occur within a few hours or days, but occasionally not until the 2nd or 3rd week. Several erythroblastotic infants have been described whose deciduous teeth were deeply discolored with a grayish-green pigment which seemed to be incorporated in the deeper layers of the teeth rather than merely upon their surface. This discoloration probably was a part of the erythroblastotic process, caused by an excessive deposit of blood pigments during the prenatal formation of the enamel and dentine.

Erythroblastosis fetalis has been classified into three distinct entities: (*a*) congenital anemia; (*b*) icterus gravis, and (*c*) fetal hydrops. Congenitally anemic infants, as a whole, have a very mild anemia at the onset which slowly becomes severe; the other two types also show a great degree of anemia from birth. The icterus gravis group is most likely to show a hemorrhagic tendency; the hydrops infants are universally severely damaged. All three groups exhibit splenomegaly and hepatomegaly. Congenital anemia is the least severe form of erythroblastosis, while hydrops is the most severe. The majority of the simple congenital anemia group recover following adequate treatment; a smaller percentage of the icterus gravis group survive; but none of the hydrops infants live. It is interesting to note that the same mother may give birth to infants manifesting any one of the three forms of the disease. The usual sequence is to find a progression in the severity of the disease as the number of pregnancies increases. The first erythroblastotic child may suffer from the congenital anemic form, the next may have icterus gravis, and the third may be a hydrops case.

As stated by Davidsohn,[5] there are four conditions which determine the severity of fetal erythroblastosis and the place where it appears in a family:

1. The age of the fetus when Rh antibodies begin to act on it.
2. The length of time the fetus is exposed to these Rh antibodies.
3. The strength of the Rh antibodies. The titer of anti-Rh agglutinins in the blood of the mother may or may not be a measure.
4. The permeability of the placenta. There may be quantitative differences of permeability in women. The permeability of the placenta may vary at different times during pregnancy.

The characteristic gross and microscopic changes in the placentas of mothers of erythroblastotic infants have been described by Hellman, Hertig and Javert.[12] The placentas may reveal hematomas which contain numerous normoblasts and erythroblasts most likely of fetal origin. Such placental lesions provide for the transmission of the Rh agglutinogen from the fetus to the mother. This results in isoimmunization of the latter.

Yannet[31] observed that certain children who had recovered from icterus gravis subsequently exhibited evidence of central nervous system injury. This included severe mental deficiency, extrapyramidal spasticity and athetosis. A necropsy on 1 of these children confirmed the relationship of the cerebral changes originally described as kernicterus with the aforementioned clinical picture.

Since Rh isoimmunization is important in the etiology of erythroblastosis

fetalis, and since kernicterus is found primarily in children with erythroblastosis, it would appear that the cerebral changes which occur in association with kernicterus may in some way be related to the Rh factor. Yannet suggests that certain cases of imbecility and idiocy now classified as of unknown etiology may arise as the result of Rh isoimmunization of the parent. In an unselected group of women with unclassified mentally deficient offspring approximately 25% were Rh negative. Among an equal number of mothers of Mongolian, diplegic and microcephalic children the incidence of Rh negative blood was in the normally expected range of about 12%. Although the total number of deficient children examined to date (approximately 100) is not large enough from which to draw definite conclusions regarding the relationship between erythroblastosis fetalis and mental deficiency, the results are of sufficient interest to warrant a further study of this subject.

The Occurrence of Erythroblastosis Fetalis. Erythroblastosis fetalis afflicts the offspring of about 1 in 400 mothers who are Rh negative.[12] Potter[23] states that about 12% of marriages are between Rh negative women and Rh positive men; and, although erythroblastosis fetalis is possible in the offspring of 12% of marriages, it occurs in only 0.1% of marriages. Schwartz and Levine[25] noted among 152 consecutive stillbirths and neonatal deaths, an incidence of erythroblastosis of 4.4 to 8.2%. Javert[13] found that 92% of mothers of erythroblastotic infants are multiparas and that the incidence of erythroblastosis varies with the parity. For example, the incidence of erythroblastosis fetalis in families in which the disease appears for the first time, in the second child or later, is about 50% for the second and subsequent pregnancies; whereas, if the first-born had the disease, nearly 100% of the subsequent ones suffer from it.[12]

At the Hospital of the University of Pennsylvania among 836 consecutive registered ward cases there were 60 Rh negative mothers; but only 4 of these patients gave birth to erythroblastotic infants, an incidence of about 6.4%. Among the 60 tested husbands of these 60 Rh negative wives, only 5 were found to be Rh negative. Table 1 shows these 4 cases of erythroblastosis fetalis and the order in which they appear in each family.

TABLE 1.—INCIDENCE OF KNOWN TRANSFUSION REACTIONS AND OF ERYTHROBLASTOSIS AMONG 60 COUPLES IN WHICH THE MOTHERS WERE RH NEGATIVE

Family ident. No.	Mothers			Infants		
				Erythroblastosis		
	Age	Present parity	Transfusions	Birth order	Form	Survival
1	30	VII	5th pregnancy—severe reaction	6	Icterus	No
				7	H.E.	Yes
2	31	X	7th pregnancy—mild reaction	8	H.E.	Yes
				9	H.E.	Yes
				10	Icterus	No
3	38	III	None	3	H.E.	Yes
4	29	II	None	2	H.E.	Yes

H.E.—Hemolytic anemia.

Note that the 2 mothers who had transfusions experienced untoward reactions to this type of treatment and that both of these women gave birth to erythroblastotic infants in their subsequent pregnancies.

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It can be seen, therefore, that the mere presence of the Rh negative factor in the woman and the Rh positive factor in the man are not always sufficient to result in erythroblastosis in the offspring. Some of the following explanations have been offered:

1. Due to the small size of many families, the chance of isoimmunization by successive pregnancies is reduced.
2. Variation in the permeability of the placenta of different individuals may influence the passage both of the Rh positive factor from the fetus to the mother and of specific antibodies from mother to fetus.
3. The ability of Rh negative women to produce immune anti-Rh agglutinins, in response to the stimulus of Rh positive cells, is variable.
3. The disease may occur in mild form and not be recognized.
4. Many males are heterozygous and therefore do not transmit the factor to all children.

Treatment of Erythroblastotic Infants. Treatment depends entirely on the condition of the infant at the time of delivery. If the child is apneic and cyanotic, intratracheal or intranasal oxygen is administered. A red blood count is taken immediately; and, if it is 3.5 million or more, no transfusion is given. The count should be repeated daily. If it does not drop during the next 5 days, no transfusion is required. However, if the original count is lower than 3.5 million or drops during the succeeding days, transfusions of Rh negative blood should be given.[2]

Gimson[9] has shown that the transfusion of Rh negative blood is better than that of Rh positive blood. Hemolysis of the patient's red cells is not prevented by giving Rh negative blood, but blood is being provided which will not be destroyed more rapidly than normally. The infant can live, by receiving such transfusions, until the hemolytic process of the disease has come to an end. Rh negative erythrocytes are known to survive at least 90 days, whereas Rh positive ones are destroyed within a few days of transfusion. The amount of blood used for transfusing these small infants was calculated by Gimson as follows:

$$\frac{\%\text{ rise of Hb required}}{100} \times \text{blood vol.}$$

The amount of blood is usually around 60 cc., and the number of transfusions varies from 2 to 6.

Heterozygous vs. Homozygous Parentage. Males who have inherited the Rh factor from only 1 parent are heterozygous and, therefore, do not transmit this factor to all of their offspring. The children who do not inherit the Rh factor escape erythroblastosis fetalis. Most fathers of erythroblastotic infants are homozygous. This fact reduces considerably the possible incidence of the disease since only about 43% of all Rh positive persons are homozygous. It is only recently that it has been possible to determine whether the father is heterozygous or homozygous.

In order to make this distinction the red blood cells of the father are set up against the three standard Rh sera—Rh′, Rh″, Rh_0—and also the Hr serum. If the father's cells are agglutinated by Rh′ and Rh_0 serum but not by the other 2, he is homozygous. He will not have any negative genes to pass on if his cells are agglutinated in Rh′, Rh″, and Rh_0 sera. If his cells are agglutinated in Rh′, Rh_0, and Hr serum, he is probably heterozygous but may belong to a rare subgroup of the Rh series. He will be heterozygous also if his cells are agglutinated in Rh_0, Rh″, and Hr serums, unless he again belongs to one of the rare subtypes.

There are some additional aids which help in this diagnosis. For

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example, if an Rh negative mother and an Rh positive father have an Rh negative infant, the father probably will be heterozygous. If either parent of the father is found to be Rh negative, the father undoubtedly will be heterozygous.

The Agglutinating and the Blocking Antibodies. As soon as a pregnant woman is discovered to be Rh negative, it is important to test for the presence of antibodies in her blood. These may exist either as agglutinating or blocking antibodies; the presence of the latter gives a much poorer prognosis for the fetus than the presence of the agglutinating antibody. Serial dilutions of the mother's serum are tested against the cells which she agglutinates. The presence of agglutinins is found by titrating against the cells most strongly agglutinated by the above procedure. The presence of the blocking antibody is tested by the method which was recently described by A. S. Wiener.[28] This antibody interferes with the agglutination of Rh positive cells by standard anti-Rh serum.

The finding of agglutinating antibodies in a pregnant woman means that there exists a potential danger to her infant. Titers of her agglutinating antibodies should be made at 6 week intervals during the first 7 months of her pregnancy and at 4 week intervals during the last 2 months. As this titer rises, the danger of erythroblastosis fetalis occurring increases. If the titer drops sharply, it can be assumed with a high degree of certainty that the fetus will be seriously affected. If the blocking antibody makes its first appearance as the titer of the agglutinating antibody falls, a fatal prognosis for the infant can be made in practically every case.

The effect of the agglutinating and blocking antibodies upon the fetus *in utero* depends upon the length of time it is exposed to these antibodies. The longer the exposure the more will be the damage to the offspring. At present some investigators think that the early appearance of a low titer is as damaging to the infant as a sudden rise in the titer in the last few months of pregnancy. The exposure of the fetus to these antibodies can be shortened by a premature delivery by means of Cæsarean section. This should be done at the time when the titer falls and before the blocking antibody appears.

The Significance of the Rh Factor in Pregnancy. Knowledge of the Rh factor is important from several clinical points of view. Rh negative women who have, in the course of a pregnancy, developed anti-Rh agglutinins may experience serious transfusion reactions which occasionally may be fatal. These reactions may occur from the first but, more frequently, from subsequent transfusions.[6] For this reason it is important to determine the Rh reaction of every pregnant woman since she may require a transfusion at the time of delivery. Knowledge of the Rh factor is important also because the transfusing of an Rh negative woman with Rh positive blood may initiate the formation of anti-Rh agglutinins and thus induce erythroblastosis fetalis in subsequent offspring. Such children may show varying degrees of hemolytic anemia.

An Rh negative woman should be tested next for the presence of any antibodies she might possess. It is important to determine if such antibodies are of the agglutinating or blocking variety. If the former are found, the titer should be determined and be followed as noted above.

The next step is to discover whether the father is homozygous or heterozygous. If the mother has had a previous erythroblastotic infant and her husband is homozygous and she now possesses agglutinins, she should be advised against having future children. However, if her husband is heterozygous, there is a better chance that normal infants may result.

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At the present time we have no method for preventing the development of erythroblastosis fetalis, but experience to date has supplied us with certain useful information. First, it is known that we can avoid sensitizing mothers who are Rh negative by transfusing them always with Rh negative blood. Second, we can predict with a relative degree of accuracy the health of offspring to be born to parents whose Rh factors differ. With such knowledge preparations for the proper treatment of the mother and child can be made in advance.

Prevention of erythroblastosis fetalis can be sought only in prophylactic directions, and may be so important to the parents as to be considered in some instances. Prevention of subsequent pregnancies by sterilization is to be considered. Also, it has been suggested that individuals prior to marriage should be tested for their Rh factor so that they may be warned in advance of the type of offspring which may result from such unions. Artificial insemination might be discussed with persons whose infants all have had erythroblastosis, but who are strongly desirous of having normal children. Therapeutic abortion also should be considered where investigation has shown that it would be unlikely for the mother to deliver a normal infant. This should be considered because the mother is not wholly without danger while carrying a sensitized infant. It has been shown also that late abortions and abruptions of the placenta occur unusually frequently in these cases.

Summary. 1. Blood transfusion reactions may occur in persons whose blood has been properly typed and cross-matched, but who receive blood of the wrong Rh type.

2. Most blood transfusion reactions in Rh negative individuals can be prevented by transfusing them always with Rh negative blood.

3. An Rh negative pregnant woman may be sensitized either by a transfusion of Rh positive blood or by carrying an Rh positive fetus. In such an event the infant is very likely to suffer from erythroblastosis fetalis.

4. If an Rh negative pregnant woman possesses both agglutinating and blocking antibodies in her serum and her husband is homozygous, great consideration should be given to the advisability of interrupting her pregnancy because of the added dangers to herself and the unlikelihood of having a normal, healthy infant. Future pregnancies also should be advised against in this case. If no agglutinins are present and her husband is heterozygous, there is a 50% chance that her infant will be normal.

5. The incidence of erythroblastotic infants born at the Hospital of the University of Pennsylvania to 60 couples in which the mother was Rh negative and the father Rh positive in 1 year was 6.4%.

6. Many infants suffering from hemolytic disease of the newborn can be saved if the occurrence of the disease is foreseen, its existence recognized at once, and the infant transfused with Rh negative blood when needed.

7. Erythroblastosis fetalis can be prevented by: (*a*) knowledge of the couple's Rh factor before marriage, (*b*) artificial insemination, (*c*) therapeutic abortion, or (*d*) sterilization.

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PHYSIOLOGY

PROCEEDINGS OF
THE PHYSIOLOGICAL SOCIETY OF PHILADELPHIA
SESSION OF APRIL 17, 1945

Factors Influencing the Maintenance of Blood Levels of Sulfonamides. R. H. SILBER and I. CLARK (Merck Institute for Therapeutic Research, Rahway, N. J.). A comparison of the fate of sulfaquinoxaline (SQ), sulfamerazine (SM), sulfadiazine (SD), and 3 derivatives of sulfaquinoxaline was made in rats after administration of single doses of 200 mg. per kg. orally or intravenously. Blood and plasma levels, liver levels, gastro-intestinal content, urinary and fecal excretion, and binding by plasma protein were determined.

Sulfaquinoxaline, an amino derivative (2-sulfanilamido-6-aminoquinoxaline), and an acetyl derivative (N_4-acetyl-sulfaquinoxaline) were 88.5 to 93.5% bound by rat plasma, compared with 74.5% SM and 62% SD. The 2 derivatives of SQ were removed from the blood as quickly as SM or SD in spite of the high degree of plasma binding. Striking prolongation of SQ blood levels resulted from excretion into the intestine and a delay in gastro-intestinal emptying time. After 24 hours the delaying effect of SQ was confirmed by the presence of 30% of an oral dose in the stomachs of fasting rats, whereas SM and SD had been completely removed. The hydroxy derivative (2-sulfanilamido-3-hydroxyquinoxaline), an insoluble compound, was also retained in the gastro-intestinal tract and after intravenous administration blood levels were prolonged almost as long as those of SQ. It was possible to maintain SD levels as well as those of SQ by withholding food after oral administration of the drug. This was correlated with a decrease in fecal loss and retention of drug in the intestinal tract.

Plasma binding alone cannot account for prolonged blood levels of sulfonamides. Other factors which determine the magnitude and maintenance of blood levels in rats are: (1) solubility in (and absorption from) the intestinal tract; (2) the presence or absence of food; (3) the emptying time of the gastro-intestinal tract; (4) excretion into the intestinal tract; and (5) urinary and fecal excretion.

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Effects Produced in Man by Inhalation of High Concentration of Oxygen for 24 Hours.* JULIUS H. COMROE, JR., ROBERT D. DRIPPS, PAUL R. DUMKE and MARGO DEMING (Depts. of Pharmacology and Anesthesia and Harrison Dept. of Surgical Research, Univ. of Penna.). The effects produced by breathing high concentrations of oxygen for prolonged periods of time have been studied by many investigators in a variety of animals and all have reported the occurrence of irritation, congestion and edema of the lungs and even death following long exposures. Similar well-controlled experiments have not yet been done upon large series of men. In the present study 100% oxygen was administered continuously to 34 healthy young males for a period of 24 hours without any intermission. Twenty-eight subjects (82%) experienced substernal aching which was usually made worse by deep inspiration. This substernal discomfort was slight in 4, of moderate intensity in 18, and severe in 6 subjects. The symptoms developed in an average of 14 hours; after the 24 hour period was completed the symptoms gradually decreased over a 4 to 12 hour period. Signs of nose, throat and eye irritation were common. The vital capacity was decreased markedly in a large number of these subjects, the maximal being 1480 cc. in 2 instances. A group of 10 subjects breathing through identical apparatus (full face mask and demand apparatus) but supplied with air rather than 100% oxygen developed no symptoms during the 24 hour period. Another group of 10 subjects breathing 50% oxygen for 24 hours developed no substernal distress. When 75% oxygen was breathed for 24 hours substernal distress was noted in 55% of the subjects. It appears that oxygen in excess of 50% is required in order to produce toxic symptoms in man over this period of time. Breathing of 100% oxygen at 18,000 ft. equivalent altitude for 24 hours produced no symptoms in a group of 6. Since the total oxygen tension in this experiment is equivalent to the inhalation of 50% oxygen at sea level, this indicates that symptoms are due to high oxygen tension rather than to elimination of nitrogen from the body.

The Effect of Respiratory Movements Upon the Circulation. ISAAC STARR and CARL K. FRIEDLAND (Hartzell Research Department of Therapeutics, Univ. of Penna.). The ballistocardiogram regularly shows a respiratory variation. The impacts increase during inspiration and diminish during expiration. If the breath is held this variation disappears. These findings fit with the concept that inspiration increases cardiac output by increasing filling pressure, and they are consistent with the results of animal experiments by Boyd and Patras (1941). However, the respiratory variation of the peripheral blood flow, as suggested by records from finger plethysmographs (Bolton *et al.*, 1936; Burton, 1939; and Burch *et al.*, 1942), was often the reverse of the ballistocardiograph changes, as were blood pressure changes in some of our patients. Also there was a possible alternative explanation of the ballistocardiograph respiratory variation, namely, that it was due to change in position of the heart rather than change of output.

To ascertain whether position or pressure changes caused the ballistocardiogram's respiratory variations, we performed 2 types of experiments. Changing the position of the subject on the ballistocardiograph, to get the direction of recording in line with the cardiac axis at full expiration, *did not* reverse the normal respiratory variation. Also in a patient in

* Work done under contract with the Office of Scientific Research and Development.

the decerebrate condition after an unsuccessful brain operation positive pressure respiration *did* reverse the normal ballistocardiograph respiratory variation. These findings demonstrate that the respiratory variation is related to pressure changes and not to position.

The opportunity to study the subject further came with the discovery of a patient with ventricular aneurism whose ballistocardiogram showed a doubled J wave. We believe that the first peak was derived from the impact from the normal right heart, while the second was from the abnormal left, which was forced to expand the aneurism before ejection. These 2 peaks had a different respiratory variation, the first increased in height during inspiration while the second diminished.

We also experimented with healthy persons given artificial respiration with blasts of air, while pressure in the mask was recorded by a Frank capsule and chest position by an inflated bag and tambour. In 1 case, arterial pressure was also recorded by a Hamilton manometer.

All our results are consistent with the following hypothesis, an old view given by Bazett (in Macleod's "Physiology in Modern Medicine," 1941) and supported by the experiments of Shuler *et al.* (1943) and Dupee and Johnson (1944). During inspiration better filling increases the output of the right ventricle but the increment first goes to fill the lungs whose blood capacity is increasing as they expand. For the same reason the left ventricle is less well filled during early inspiration and its output diminishes; later, if inspiration is sufficiently prolonged, the wave of increased output from the right side reaches the left and increases its output. During expiration right ventricular filling diminishes, and its output diminishes also. But now blood is squeezed from the contracting lungs into the left side of the heart and its output is maintained or increases.

Healthy subjects with sinus arrhythmia show an interesting phenomenon when given artificial respiration with blasts of air. The slowing of the pulse, normally present during expiration, then appears at other positions of the respiratory cycle, especially during inspiration. Under all circumstances it is the small impacts that are slowed. The heart slows down as if it were waiting to be properly filled before contracting.

Treatment of Clinical Hyper-pituitarism With Hypertension by Injections of Pitressin Tannate in Oil. J. Q. Griffith, Jr., N. Padis, and E. Anthony (Robinette Foundation, Hospital of the University of Pennsylvania). Sixty-two patients were studied who had both elevated arterial pressure and a blood serum that was able to delay water diuresis in rats when injected intraperitoneally. There were 33 males and 29 females. Ages ranged from the 2nd to the 6th decade, with only 5 subjects below 30. Period of observation ranged from 3 to 26 months following treatment, the average being 8 months. In all, 63 courses of treatment were given: (1) 13 persons were given injections of aqueous pitressin (Parke Davis) hypodermically, the dose ranging from 0.2 to 1 cc., repeated daily or at longer intervals, for 6 injections or more; (2) 10 persons were given 1 cc. of pitressin tannate in oil (Parke Davis) at weekly or monthly intervals, the number of injections being less than 6; (3) 40 persons were treated similarly, except the injections were 6 or more. Results were definitely better for the last group than for the first 2 groups. However, considering all 3 groups as a whole, systolic and diastolic blood pressures were significantly lowered and clinical improvement occurred in about 50% of

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the subjects. This was associated, usually, with disappearance of antidiuretic substance from the patient's serum. Results could not be predicted on the basis of age or sex, nor from preceding testing of cutaneous lymphatic flow, or capillary elasticity as demonstrated by cutaneous capillaroscopy. No patients were treated who showed evidence of diminished renal function, or had high titers for gonadotropic hormone in serum. Sharp reactions occurred following the injections of the aqueous preparation, but not after the preparation in oil, although 2 subjects showed mild and transient urticaria. Injection therapy was not used in the very elderly and especially not in those with systolic blood pressure much above 200. We, therefore, suggest that repeated injections of pitressin tannate in oil (not aqueous pitressin) offer a form of therapy effective in certain selected cases of hypertension.

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BOOK REVIEWS AND NOTICES

THE MARCH OF MEDICINE. The New York Academy of Medicine Lectures to the Laity, 1944. Pp. 121. New York: Columbia Univ. Press, 1945. Price, $1.75.

AMONG the many enviable achievements and activities of the New York Academy of Medicine, its series of lectures to the laity, now in its 10th year, is one of the most significant. These lectures aim to reveal to the public the historical and philosophic backgrounds of medicine and public health, and their relationships with the collateral sciences of psychology, sociology, criminology, economics, and even world politics—a considerably larger target than merely to keep the public informed about recent medical discoveries. They are no small tribute to the vision of the Academy's benefactor, Linsley Williams, after whom one of the lectures is appropriately named.

The 6 lectures of the present series are, as usual, all by eminent men and cover an even wider range than cabbages and kings: Morale and Propaganda (E. A. Strecker); Food and Civilization (C. G. King); The Past, Present, and Future of Chemotherapy (C. M. MacLeod); Medicine and the Changing World (R. Fitz); The Effects of Science Upon Human Beings (Sir G. Campbell); and Wars and Epidemics (Lt. Col. T. T. Mackie). The series, labelled by Dr. A. F. Chace, the Academy's president, "War and the Expanding Frontiers of Medicine" gives pabulum to many kinds of mind and taste. Dull indeed is the mind or taste that finds no profit or pleasure in the perusal of this volume of the March of Medicine. E. K.

THE COMMON COLD AND HOW TO FIGHT IT. By NOAH D. FABRICANT, M.D. Pp. 107. New York: Ziff-Davis, 1945. Price, $1.50.

THIS little book, written by a nose and throat specialist, is a comprehensive survey of all that is currently known concerning the common cold. Though intended primarily for the intelligent layman, the material differs from that which might be found in a medical textbook only in the simplicity and clarity of presentation.

After a preliminary description of the structure and function of the nose Dr. Fabricant surveys our present knowledge concerning the causes, complications, prevention and treatment of the cold. After reviewing the many methods used for treating the cold and indicating their questionable value, the author states that warmth, rest in bed, and the application of moisture to the nose are the sanest techniques available.

More specific therapy will have to await the work of the research man.

M. K.

A TEXT BOOK OF PSYCHIATRY FOR STUDENTS AND PRACTITIONERS. By D. K. HENDERSON, Professor of Psychiatry, Univ. of Edinburgh, and R. D. GILLESPIE, Physician for Psychological Medicine, Guy's Hosp., London. Sixth Ed. Pp. 719. New York: Oxford Univ. Press, 1944.

THIS edition maintains the high standard of previous editions, which, in the Reviewer's opinion, have established the work as the most satisfactory text available in English. In addition to incorporating the important recent advances, the latest edition presents some minor rearrangements of contents. The section on the psycho-neurotic reaction types now precedes that on the

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psychoses and all the paranoid reaction types have been grouped together. A chapter on "Special Methods of Physical Therapy" summarizes excellently the present status and techniques of the various shock therapies. A short discussion of the electro-encephalographic findings has been included in the chapter on "Epilepsy." The section on "War Psychiatry," revised and enlarged, presents a basic survey of the problems encountered in this important aspect of Psychiatry. In line with current trends, the social aspects of Psychiatry have been stressed. The general orientation follows the psychobiologic approach of Adolph Meyer.

This book is highly recommended to students, practitioners, and others as a comprehensive, sound, and very readable authoritative text on Psychiatry.

C. R.

NEURO-OPHTHALMOLOGY. By DONALD J. LYLE, B.S., M.D., F.A.C.S., Lecturer on Neuro-ophthalmology, Department of Anatomy, Med. Coll. of the Univ. of Cincinnati; Attending Ophthalmologist to the Good Samaritan Hosp., Christ Hosp., Jewish Hosp., St. Mary's Hosp., and Children's Hosp. Pp. 398; 7 charts, 529 ills. Springfield, Ill.: Thomas, 1945. Price, $10.50.

THIS is a welcome addition to the few monographs in English which deal primarily with Neuro-ophthalmology. It is more of an introduction than a handbook and the graduate student will naturally desire much more information than this book affords.

The illustrations are good and as far as it goes, the text is readable and well documented with case reports. A very excellent bibliography of some 1700 references is given at the end of the book.

F. A.

THE BIOLOGICAL BASIS OF INDIVIDUALITY. By LEO LOEB, Professor Emeritus of Pathology, Washington Univ. School of Medicine, St. Louis. Pp. 714; no ills. Springfield, Ill.: Thomas, 1944. Price, $10.50.

THE purpose of this book is to analyze the foundations of individuality. Two types of individuality are considered: "The first one is the mosaic type which represents the sum of the particular organ and tissue characteristics (organ and tissue differentials) which determine structure, metabolism, motor and psychic activities and the component parts of which differ in different individuals." "The second type of individuality which may be designated as the essential individuality is characterized by the presence of a chemical factor—which is common to the different organs and tissues of each individual and which differs from the corresponding chemical characteristics of the organs and tissues of every other individual."

The book is arranged in 8 parts, with chapter subdivision. Part I, Transplantation of Tissues in Higher Vertebrates, as a Method for the Analysis of the Organismal Differentials. Part II. The Phylogenetic and Ontogenetic Development of Individuality and Organismal Differentials. Part III. The Significance of Organismal Differentials in the Interaction Between Single Cells. Part IV. Tumors and Organismal Differentials. Part V. Organismal and Organ Differentials and the Specificity of Tissue Reactions. Part VI. Organismal Differentials and Organ Differentials as Antigens. Part VII. Organismal Differentials, Organ Differentials and Evolution. Part VIII. The Psychical—Social Individuality.

The material is drawn largely from observations of the host to transplanted tissues from various sources, a field in which the author has published many papers. The Reviewer found the book somewhat difficult to read because of the author's style, but was impressed by the vast amount of material and the sound reasoning employed in its interpretation. The comprehensive bibliography should be of value to workers in almost any branch of biologic investigation. It is difficult to evaluate the book from the standpoint of its contribution to our knowledge of Biology. To the worker who employs transplantation methods the volume holds a wealth of material.

D. C.

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THE PATHOLOGY OF INTERNAL DISEASES. By WILLIAM BOYD, M.D., LL.D., M.R.C.P. (EDIN.), F.R.C.P. (LOND.), DIPL. PSYCH., F.R.S.C., Professor of Pathology and Bacteriology in the Univ. of Toronto. Fourth Ed., revised. Pp. 857; 366 engr., 8 color plates. Philadelphia: Lea & Febiger, 1944. Price, $10.00.

Early in its career this textbook established a well-deserved reputation as the most easily readable of the pathology textbooks. Thus, it soon became a favorite of students. Now it is in the 4th edition and is still going strong.

In this new edition there are no radical changes but a number of important additions have been made. New material includes: the demonstration of collateral coronary circulation by injection, the relation of trauma to coronary disease, disseminated lupus erythematosus, atypical pneumonia, psittacosis, adenomatosis of the lung, blast injury, infectious hepatitis, dietary cirrhosis, alloxan diabetes, intercapillary glomerulofibrosis (sclerosis), nephrosis following incompatible transfusions and crush injury, the pathogenesis of Graves' disease, sarcoidosis, and the importance of the Rh factor in erythroblastosis.

A few sections have been largely rewritten, notably etiology of rheumatic fever, the time factor in coronary infarcts, pyelonephritis, intracranial aneurism, and the pathogenesis of poliomyelitis.

There are several new figures and color plates.

This new edition will undoubtedly maintain its place as one of the leaders among the standard pathology textbooks and can be recommended for both student and practitioners. W. S.

THE NEUROLOGIST'S POINT OF VIEW: Essays on Psychiatric and Other Subjects. By I. S. WECHSLER, M.D. Pp. 251. New York: Fischer, 1945. Price, $3.00.

THIS author states that the Jew has a neurotic character makeup, and that persisting to be a Jew means to court the possibility of a neurosis; but, he continues: "among all peoples and races whatever progress was made came from men of neurotic makeup, if not with actual neuroses." The prejudices and conflicts which harm our individual lives, and the origins of modern man's difficulties in adjustment are among the important topics discussed; these and other subjects constitute this collection of papers published previously. The chapters are: Nervousness and the Jew; An Inquiry Into Racial Psychology; The Legend of the Prevention of Mental Diseases; The Neurotic Conflict Between the Individual and Society; The Psychology of Anti-Semitism; Anti-Semitism as a Neurosis; Sigmund Freud: a Critical Appreciation; The Problem of Mental Disorders: The Neurologist's Point of View; Maimonides the Physician; Moses and Monotheism: A Review; On Palestinian and Russian Colonization, A Chronicle; A Brief History of Psychiatry.

In regard to Freud, doubt is expressed as to whether objective criticism is yet permissible. While admitting the danger of great generalizations on isolated observations, Freud is said to have possessed the essentials necessary to a scientist or poet—great ingenuity and wide imagination to a hazardous degree. Freud's concept of the unconscious is regarded as his greatest contribution, wherein is visioned "an active, dynamic unconsciousness which is entirely outside the realm of awareness and which, indirectly, is capable of influencing conscious behavior." The "nonsense" of Adler and the "universal consciousness" of Jung are considered outside the realm of psychoanalysis. Freud's fine concept of sublimation has been assailed in "that it does violence to ethical principles and noble ideals to allege that they are rooted in ugly unconscious impulses." Plunging into this controversy, the author says: "Friendship is a beautiful sentiment, even though it be alleged that it is the sublimated result of unconscious infantile homosexuality." Among other conclusions the writer states: "I would venture the guess that analysis will be remembered longest for the insight into normal and abnormal behavior which it has vouchsafed and for its excellence as a method of investigation." Freud's effort to establish Moses as an Egyptian, or to question his

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ever having lived, was not pleasing to most Jewish people, nor have his contentions convinced most scholars. That the Jews were the traditional "Chosen People," was accepted by Freud, but he asserts it was Moses who chose them.

N. Y.

CONSTITUTION AND DISEASE. Applied Constitutional Pathology. By JULIUS BAUER, M.D., Professor of Clinical Medicine, Coll. of Medical Evangelists, Los Angeles. Pp. 247. 2nd Ed. New York: Grune & Stratton, 1945. Price, $4.00.

THE author appears to define "applied constitutional pathology" as the facts of endocrinology and other branches of medicine correlated to the particular situation encountered in individual patients. Yet I can read through large sections of this book without finding any such correlation. Surprise is not limited to the obvious shortcomings in the interpretation, it is equally unexpected to hear from Germanic sources that Americans neglect the patient's whole personality in their concern about laboratory procedures and should give heed to the artistic trend of pre-war Vienna and Paris. It is a poor practitioner, indeed, who would not subscribe to the aphorism "In clinical medicine the laboratory is a good servant but a bad master." The author endorses the general dislike of speculative approach insufficiently supported by facts, yet many statements in a text fall into this error. These of course are serious not fatal shortcomings in a book "intended to familiarize the reader with the principles of constitutional thinking"; and one may say that this aim has been accomplished. Furthermore, numerous examples of the importance of the constitution are to be found and valuable advice offered on such matters as the range of normality. One is glad to find an index to this second edition.

E. K.

THE DOCTOR'S JOB. By CARL BINGER, M.D. Pp. 243. New York: Norton, 1945. Price, $3.00.

THE layman for whom these essays appear to have been written—or is it perhaps the young medico starting on his career, or the older medico needing to adapt to the changing times?—will find much of interest and value between these covers. The reader will quickly discover about the author—what the well-informed American internist already knows—that his keenly observing, sensitive nature has been fostered by an excellent medical education—both before and after graduation—and leavened by his years of practice in New York City. Pleasant glimpses of his love of the sea and of music and the arts are frequent. Incidentally, the rather hard, calculating physiognomy portrayed on the jacket is, to the Reviewer at least, an unfortunate libel on Dr. Binger's sensitive, intellectual, yes, handsome features. In 18 more or less connected chapters, the author interprets the changes that have come over medicine—the expansion of medical science; the disappearance of "the encyclopedic wise man" ("It is only a few years since doctors came out from behind the ambush of their beards"); growth of the specialties; the socialization of medicine; psychoanalysis and psychosomatic medicine (an irritating word for something that the real doc has always practiced!); and some of the popular diseases of our time. Much is being written on these matters—tripe and otherwise—but one must go far to find more penetrating, wiser comments than appear throughout this book. To be sure, "I" might appear less often in the text to advantage; racial discrimination looms over-prominently; the belief that the general practitioner is on the way out will be denied by many; and the author has fallen into the frequent error of intimating that reform in medical education in the U. S. really owes all to the founding of Johns Hopkins and to Abraham Flexner's report. But these are minor criticisms that are soon effaced by the torrent of fundamental wisdom, set off by apt quotations from Paracelsus and others and by the author's own trenchant observations: "the secret of the care of the patient is in caring for the patient" (F. W. P.);

"he who wants to know man must look upon him as a whole" (P. A. T. B. von H.); the internist is "the conductor of the symphony who calls out the oboe and the tympanum and does not let either steal the show" (C. B.); as to the need for choosing a good doctor "a *pretty* good doctor is like a *pretty* good egg" (George Sears); "the patient should be in more or less continuous contact with a doctor whom he knows and trusts . . . . and stick to him and take his advice until he is done with him and then choose another. . . . . It is far more important that he possess integrity" (than brilliance) (C. B.); "When a clinic record reaches a certain thickness, a certain weight, the patient is usually referred for a psychiatric consultation" (H. B. R. in *Patients Have Families*); "We must have more doctors trained in both medicine and psychiatry. . . . . There is almost no department of human behavior that does not fall into the province of the psychiatrist's interest" (C. B.); "Like happiness a cure is seldom found by searching for it" (C. B.). In a final paragraph we find among the obstacles to the good physician's task: "Our patients demand miracles of us. They limit our moves by making pawns of us—or kings—when we should range freely as knights. . . . . These (our professional standards) we fight for gladly. It is indeed a poor heart that never rejoices."

E. K.

NEW BOOKS

The Medical Clinics of North America. Symposium on New Developments in Medicine. Pp. 561; 96 figs. Phila.: Saunders, 1945. $16 per year.

On Modern Syphilotherapy. With Particular Reference to Salvarsan. By ALBERT NEISSER. Translated by ISABELLE VON SAZENHOFEN WARTENBERG. Pp. 42. Baltimore: Johns Hopkins Press, 1945. Price, $1.00.

Proceedings of the Rudolf Virchow Medical Society in the City of New York. Pp. 103. Vol. III, 1944. New York: Brooklyn Medical Press, 1945. Price, $2.00.

Mass Radiography of the Chest. By HERMAN E. HILLEBOE, M.D., Medical Director, Chief, Tuberculosis Control Division, United States Public Health Service; Professorial Lecturer on Tuberculosis Control, George Washington Univ. School of Medicine, Washington, D. C., and RUSSELL H. MORGAN, M.D., Surgeon (R), Medical Officer-in-Charge, Radiology Section, Tuberculosis Control Division, United States Public Health Service. Pp. 288; 93 figs. Chicago: Year Book Publishers, 1945. Price, $3.50.

Malaria in the Upper Mississippi Valley (1760–1900) (Supplements to the Bulletin of the History of Medicine). By ERWIN H. ACKERKNECHT. Pp. 142. Baltimore: Johns Hopkins Press, 1945. Price, $2.00 ($1.75 to subscribers).

A Manual of Tropical Medicine. Prepared under the Auspices of the Division of Medical Sciences of the National Research Council. By COL. THOMAS T. MACKIE, M.C., A.U.S. (and 8 others). Pp. 727; 287 ills., 6 in color. Phila.: Saunders, 1945.

BRONCHIAL ASTHMA. By LEON UNGER, B.S., M.D., F.A.C.P., Assistant Professor, Department of Medicine, Northwestern Univ. Medical School, Chicago. Introduction by MORRIS FISHBEIN, M.D., Editor, *Journal of the American Medical Association.* Pp. 724; 117 ills. Baltimore: Thomas, 1945. Price, $9.00.

The New York Hospital. A History of the Psychiatric Service (1771–1936). By WILLIAM LOGIE RUSSELL, Professor of Psychiatry, Emeritus, Cornell Univ. Med. Coll., Consulting Psychiatrist, New York Hosp. Pp. 556. New York: Columbia Univ. Press, 1945. Price, $7.50.

Doctors at War. Edited by MORRIS FISHBEIN, M.D., Editor of the *Journal of the American Medical Association* and of *Hygeia,* The Health Magazine; Chief Editor of *War Medicine;* and Chairman of the Committee on Information of the Division of Medical Sciences of the National Research Council. Pp. 398; 82 photos, plus charts and diagrams and an index. New York: Dutton, 1945. Price, $5.00.

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The Examination of Reflexes. By ROBERT WARTENBERG, M.D. Foreword by FOSTER KENNEDY, M.D. Pp. 222. Chicago: Year Book Publishers, 1945. Price, $2.50.

Dietotherapy. Clinical Application of Modern Nutrition. Edited by MICHAEL G. WOHL, M.D., Associate Professor of Medicine, Temple Univ., School of Medicine; Chairman, Advisory Committee on Nutrition, Philadelphia Department of Public Health. With a Foreword by RUSSELL M. WILDER, M.D., PH.D., Professor of Medicine and Chief of the Department of Medicine, Mayo Foundation. Pp. 1029; 90 figs. Phila.: Saunders, 1945.

NEW EDITIONS

Technique of the Standard Kahn Test and of Special Kahn Procedures. By REUBEN L. KAHN, Chief of Clinical Laboratories, Univ. of Michigan Hosp. Pp. 52. Revised and Enlarged Edition. Michigan: University of Michigan, 1944. Price, $.25.

The Human Body. By LOGAN CLENDENING, M.D. 4th Ed., corrected, enlarged, and rewritten. Pp. 443; over 100 ills. New York: Knopf, 1945. Price, $4.00.

The New-born Infant. A Manual of Obstetrical Pediatrics. By EMERSON L. STONE, M.D., Associate Clinical Professor of Obstetrics and Gynecology, School of Medicine, Yale Univ.; Attending Obstetrician and Gynecologist to the New Haven Hosp. 3rd Ed., thoroughly revised. Pp. 314. Phila.: Lea & Febiger, 1945. Price, $3.25.

The Fundamentals of Electrocardiographic Interpretation. By J. BAILEY CARTER, M.D., F.A.C.P., Assistant (Rush) Professor, Department of Medicine, Univ. of Illinois Coll. of Medicine; Attending Staff, Cook County Hosp., Augustana Hosp., Chicago. 2nd Ed. Pp. 406, 307 figs. Springfield: Thomas, 1945. Price, $6.00.

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